

**“STUDIES ON SEED INOCULATION OF MPKV’S
RHIZOBIUM AND PSB LIQUID BIOFERTILIZER IN
SOYBEAN”**

By

Miss. NANAWARE PRANITA BHASKAR

Reg. No. (012/254)

A Thesis submitted to the

**MAHATMA PHULE KRISHI VIDYAPEETH,
RAHURI-413 722, DIST. AHMEDNAGAR,
MAHARASHTRA STATE (INDIA)**

In partial fulfilment of the requirements for the degree
of

MASTER OF SCIENCE (AGRICULTURE)

in

AGRICULTURAL MICROBIOLOGY

**DEPARTMENT OF PLANT PATHOLOGY AND
AGRICULTURAL MICROBIOLOGY,
POST GRADUATE INSTITUTE,
MAHATMA PHULE KRISHI VIDYAPEETH,
RAHURI-413 722. DIST. AHMEDNAGAR, M.S., INDIA.**

2014

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2014**

CANDIDATE'S DECLARATION

I hereby declare that this thesis or a part
there of has not been submitted
by me or any other person
to any other University
or Institute for
a Degree or
Diploma.

Place: MPKV, Rahuri

Date: / / 2014

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CERTIFICATE

This is to certify that the thesis entitled, “**STUDIES ON SEED INOCULATION OF MPKV’S RHIZOBIUM AND PSB LIQUID BIOFERTILIZER IN SOYBEAN**”, submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar, Maharashtra State, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (AGRICULTURE)** in **AGRICULTURAL MICROBIOLOGY** embodies the results of a bonafide research work carried out by **Miss. NANAWARE PRANITA BHASKAR**, under my guidance and supervision and that no part of the thesis has been submitted for any other degree, diploma or publication.

The assistance and help received during the course of this investigation have been acknowledged.

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Date :

Place : Rahuri

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LIST OF ABBREVIATIONS

%	: Percent
/	: Per
@	: At the rate of
°C	: Degree Centigrade
CD	: Critical Difference
CFU g ⁻¹	: Colony forming unit per
cm	: Centimeter (s)
CRYEMA	: Congored yeast extract mannitol agar
DAS	: Days after sowing
e. g	: For example
et.al.	: Et alli (and others)
etc.	: Et cetera (and so on)
Fig.	: Figure (s)
G	: Gramme (s)
ha	: hectare
i.e.	: That is (id est)
Kg	: Kilogram
Kg ha ⁻¹	: Kilogram per hectare
lit	: Liter
mg	: Milligram
ml	: Milliliter
ml ⁻¹	: Per ml
N	: Nitrogen
No.	: Number
ODg ⁻¹	: Optical density per gram
P	: Phosphorus
plant ⁻¹	: Per plant
PSB	: Phosphate solubilizing bacteria
pp	: Pages
q	: Quintal(s)
q ha ⁻¹	: Quintal per hectare
Rh	: <i>Rhizobium</i>
S.E	: Standard error
Spp.	: Species
Viz.	: Videlicet (Namely)

ABSTRACT

“STUDIES ON SEED INOCULATION OF MPKV’S *RHIZOBIUM* AND PSB LIQUID BIOFERTILIZER IN SOYBEAN”

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A candidate for the degree
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RAHURI – 413 722
2014

Research Guide	: Dr. C. D. Deokar
Department	: Plant Pathology and Agril. Microbiology

The present investigation entitled “Studies on Seed inoculation of MPKV’S *Rhizobium* and PSB liquid biofertilizer in Soybean” was conducted at field of Department of Plant Pathology and Agril. Microbiology, Post Graduate Institute, MPKV, Rahuri (Ahmednagar) -413722 in kharif season of 2013-14. The objectives of the present research were to standardize the dose of liquid biofertilizer *Rhizobium* and PSB to see the microbial population of *Rhizobium* and PSB before application and 30,60,90 days of crop growth, to study the nodulation pattern at 30,60,90 days of crop growth, to see the effect of liquid biofertilizer on crop growth and yield of soybean crop.

The observations on different growth parameters were recorded which indicated the results as follow :

1. The *Rhizobium* and PSB population were recorded higher in case of seed inoculation of MPKV'S liquid biofertilizers as compared with lignite based biofertilizer inoculation and uninoculated treatment.
2. The combined use of both *Rhizobium* and PSB liquid biofertilizers @ 25 ml each kg-1 showed greatly significant effect in case of all the growth parameters of soybean crop.
3. The number of root nodules were recorded more in case of combined (*Rhizobium* + PSB)liquid biofertilizer inoculation treatment as compared with single inoculation of liquid biofertilizer, lignite based biofertilizer inoculation and uninoculated/control treatment.
4. All the growth parameters showed their higher performance at 60 DAS of Soybean crop which clearly reflects the higher activities of *Rhizobium* and PSB microorganisms at 60 DAS of crop growth
5. This finding explicitly indicated the possibility of saving Nitrogen and phosphorus fertilizer to the extent of 25 per cent ha⁻¹.
6. The inoculation of soybean seed with *Rhizobium* and PSB liquid biofertilizer recorded the increase in yield of soybean upto 10 per cent.

From the studies it is concluded that the seed inoculation with MPKV'S *Rhizobium* and PSB liquid biofertilizers @ 25 ml kg⁻¹ of seed showed better and significant result on nodulation pattern growth and yield of soybean crop.

1. INTRODUCTION

Soybean (*Glycine max* L. Merrill) is a leguminous crop of family Leguminoceae with subfamily Papillionadae having origin in China. Soybean is basically a pulse crop but gaining importance as an oilseed crop now a days. Soybean is widely cultivated in tropical, subtropical and warm temperate regions. Optimum temperature range for most of the soybean varieties is 26.5 to 30° C.

Soybean is one of the important crop of the world. The world area under soybean is about 125 million hectares with a production of 285 million tons. Major soybean growing countries are USA, Mexico, Brazil, China and India. India ranks fifth in production after USA, Mexico, Brazil and China. In India it is cultivated on area of 9 million hectares with a production of 10.10 million tons (Source : www.faostat.fao.org). Madhya Pradesh, Maharashtra, Rajasthan and Uttar Pradesh are main growing states of soybean in our country. Madhya Pradesh is leading in production with share of 54 per cent followed by Maharashtra with 20 per cent (Source : www.sopa.org).

In Maharashtra the Area under Soybean cultivation during Kharif 2013 was 38.704 lakh hectare as compared to 32.130 lakh hectare during *Kharif* 2012. The yield 1255 Kg per hectare and production 48.565 lakh ton were estimated during Kharif 2013 (Source : www.sopa.org). Soybean cultivation is concentrated in two regions of Maharashtra, viz: Vidarbha and

Marathwada located in the eastern part of Maharashtra. Around 80 percent of the soybean production of the state is contributed by these regions. The area under the crop is highest in the former region specifically in Nagpur district. However, yield is seen to be higher for Kolhapur region located in western Maharashtra and which receives good irrigation practices (Pawar *et al.*, 2013).

Soybean is also called as nature's versatile crop is a rich source of proteins and oil. As Indian diet is deficient in proteins, both in quality and quantity, soybean can be considered as "Poor man's meat". Soybean is the largest oilseed crop of the world contributing 58 per cent in total oilseed production. Soybean contains about 40-44 per cent protein, 23 per cent carbohydrates and 20 per cent cholesterol free oil. Soybean proteins are rich source of valuable amino acid, lysine (5 %), 60 per cent polyunsaturated fatty acids (52.8 % Linoleic acid and 7.2 % Linoleic acid). Vitamins A, B, C, D, E and K are also present in soybean (Aslam *et al.*, 1995).

Application of chemical fertilizer leads to severe degradation of soil and also cause environmental pollution. So it is today's need to use environment friendly biofertilizers.

Biofertilizers are referred as the fertilizer that contains living microorganisms and it is expected that their activities will influence the soil ecosystem and produce supplementary substances for the plants (Parr *et al.* 2002). There are two types of Biofertilizers viz., carrier based

biofertilizers and liquid biofertilizers. Liquid biofertilizers have more advantages over carrier based biofertilizers so it is more beneficial to use liquid biofertilizers (Pindi and Satyanarayana, 2012).

Liquid biofertilizers are the microbial preparations containing specific beneficial microorganisms which are capable of fixing or solubilizing/mobilizing plant nutrients by their biological activity (Bhattacharya and Kumar, 2000). They have high commercial values. Liquid biofertilizers have better survival on seeds and soil and also longer shelf life up to 12 to 24 months, cost saving on carrier material, pulverization, sterilization, neutralization, packing and transport. They are also very easy to use for farmers (Chandra *et al.* 2005).

Liquid biofertilizers are grouped as Nitrogen fixing microorganism, phosphorus solubilizing microorganisms and Potash mobilizing microorganisms. Among these three groups, phosphorus solubilizing microorganisms are more extensively used compared to other biofertilizers. Liquid biofertilizer is not the usual broth culture from the fermentor or water suspension of carrier based biofertilizer, but these are liquid formulation containing not only the desired microbes and their nutrients but also special cell protectants or substances that encourages formation of resting spores or cyst for longer shelf life and tolerance to adverse conditions.

In agricultural ecosystem *Rhizobium* have vital role in fixing N₂ nutrients symbiotically (Aouani *et al.* 2001). *Rhizobium*

is a gram negative anaerobic microorganism which fix atmospheric nitrogen symbiotically with leguminous plant.

These microorganisms occur in soil naturally but their populations are often scanty. The beneficial microbial population occurring in soil may not always support for higher crop yield. In order to increase the crop yield, the desired microbes from rhizosphere are isolated and cultured in adequate quantity and mixed with suitable carrier or liquid medium. The phenomenon of strain variation in nitrogen economy presents possibilities of increasing yield of different legumes by inoculation with improved effective culture. It is therefore to isolate effective strain of *Rhizobium* from soil for increase yields through seed inoculation.

Soybean can fix nitrogen from the atmosphere if nodulated by *Rhizobium*. Nodulation requires the inoculation of the seed prior to planting with *Rhizobium* bacteria specific for soybean.

Root nodules developed by *Rhizobium* is the site of nitrogen fixation. It contains enzyme nitrogenase which helps in conversion of N_2 to NH_3 which is available form of N to plant. A characteristic feature of the healthy root nodules of leguminoids plants is the presence of a special red pigment like leghaemoglobin. *Rhizobium* can fix 50-100 kg nitrogen per crop season.

In recent years, the investigations carried out showed that soil microorganisms like bacteria and fungi play an

important role in solubilizing P in soil and making it available to plants (Wani, 1980 and Gaur, 1990). Phosphate solubilizing microorganisms as a group form an important part of the microorganisms, which benefit plant growth and development. Microbial mediated solubilization of insoluble phosphates through release of organic acids is often combined with production of other metabolites, which take part in biological control against soil born phytopathogens (Nikolay Vassilev, 2006). Soil microbes play a key role in solubilization of inorganic phosphate to release phosphorus which is available for crop growth and development (Rodriguez and Fraga, 1999). P is present in the form of H_2PO_4 and HPO_4 for the uptake by the plants; conversion from insoluble to available form is known as Mineral Phosphate Solubilization (MPS). High proportion of these phosphate solubilizing microorganisms (PSM) are concentrated in the rhizosphere of plants (Vazquez *et al.*, 2000). Many soil fungi and bacteria are known to solubilize inorganic phosphates (Illmer and Schinner, 1992).

Many common heterotrophic bacteria like *Bacillus megaterium*, *Bacillus polymyxa*, *Pseudomonas striata*, *Bacillus subtilis*, *Pseudomonas rathonis* possess the ability to convert sparingly soluble or insoluble inorganic phosphate to soluble form by secreting organic acids. The most important aspects of phosphorus cycle are microbial mineralization, solubilization and mobilization besides chemical fixation of phosphorus in soil. The phosphate solubilizing bacteria can grow in media where

$\text{Ca}_3(\text{PO}_4)_2$, FeSO_4 , AlFO_4 , apatite, bonemeal, rock phosphate or similar insoluble phosphate compounds are the sole source of phosphate. Such organisms not only assimilate the phosphorus, but also cause a large amount of soluble phosphate to be released in excess of their own requirement. Phosphate solubilizing bacteria saves P_2O_5 upto 35 to 58 kg/ha. Liquid phosphorus solubilizing bacteria formulation increases the compatibility of other beneficial microbes with plants. It also increases micronutrients with plants and soil depends upon the nutrient present in non-available form. Therefore, the present investigation entitled, “Studies on seed inoculation of MPKV’s *Rhizobium* and PSB Liquid Biofertilizer in Soybean” was undertaken with the following objectives.

Objectives:

1. To standardize the dose of *Rhizobium* and PSB liquid biofertilizer.
2. To study the microbial population of *Rhizobium* and PSB before application and 30, 60, and 90 days after sowing.
3. To study the nodulation pattern at 30, 60 and 90 days after sowing.
4. To see the effect of liquid biofertilizer on growth and yield of Soybean.

2. REVIEW OF LITERATURE

A conceptual frame work for the study based on the ideas and concepts gathered from review of existing literature facilitates the planning for studying in a comprehensive way.

The literature reviewed is presented under the following heads.

2.1 **Effect of seed inoculation with *Rhizobium* on growth and yield of soybean and other crop**

Panse and Sunkhatme (1957) reported statistical analysis by Micro-stat method.

Sundra Rao and Sinha (1963) recorded that the microbial colonies showing the transparent clear zone around their colonies.

Khandasamy and Prasad (1971) reported that growth of *Rhizobium spp.* inoculated with lignite was much better at 28 °C than at 35 °C. Highest count obtained in lignite was 13.14×10^8 which was much superior to highest count of 1×10^8 reported in soil culture.

Rewari *et al.* (1973) reported increased yield of soybean due to the inoculation of *Rhizobium* culture and increased dry weight of shoot over control.

Raut and Ghonsikar (1977) inoculated soybean seeds with *Rhizobium japonicum* and sown in rows with alternate rows of uninoculated seeds and observed that inoculated rows gave higher yields than uninoculated ones.

Sable and Khuspe (1977) reported that application of *Rhizobium* culture increased the number of pods per plant, grain and straw yield per plant and 100 grain weight over the control in soybean.

Bedmar and Olivares (1979) reported nitrogen fixation (assayed by acetylene reduction) by free-living *Rhizobium meliloti* strain while growing in a defined medium in the absence of a plant host. The highest nitrogenase activity (96.7 nmol C₂H₄ mg protein⁻¹hr⁻¹) was recorded by *R. meliloti* strain growing in defined liquid medium under normal atmosphere in sealed tubes.

Sheelavantar (1982) observed that seed inoculation with *Rhizobium* culture significantly increased the nodule number, grain yield and protein content of grain over uninoculated control in soybean [*Glycine max* (L.) Merr].

Chowdhary *et al.* (1983) revealed that *Rhizobium* inoculation significantly increased the nodulation pattern at flowering and pod filling stage in soybean. The yield was also significantly increased over uninoculated control.

Pahalwan and Tripathi (1984) confirmed that the seed bacterization by *Rhizobium* culture increased the number, dry weight and N content of nodules per plant.

Hunt *et al.* (1985) revealed that soybean seeds treated with *Rhizobium japonicum* had higher number of nodule formed, higher shoot total N-accumulation and seed yield over uninoculated plants.

Cuppucino and Sherman (1987) reported that the microbial count was calculated by multiplying the average plate count with dilution factor

Joshi (1989) reported that seed inoculation with *Rhizobium* increases the number of nodules and pods per plant and 100 seed weight, also yield increased from 0.88 to 0.97 t as compared to uninoculated in soybeans. Increasing N rates from 0-40 kg/ha increased soybean yields from 0.83 to 1.02 t and protein yield show similar results.

Maiti and Sen (1990) reported that soybean seeds inoculated with *Rhizobium japonicum* CB-1809 registered significant increase in plant height (70.6 cm), root length (15.11 cm), number of nodules (25.16/pl) and N-content (2.10 %) as compared to the uninoculated control which recorded 38.03 cm, 8.33 cm, 0 nodules/pl, and 1.40 per cent respectively.

Rehman and Sanoria (1990) evaluated the effect of nine *Bradyrhizobium japonicum* strains on growth of soybean cv. PK-327 and reported that inoculation with strains BAU-107, S-646 and TSH-501 was most effective in increasing N fixation, number of branches per plant and growth rate at different growth stages. Growth rate was positively correlated with N fixation at flowering and mid pod formation stage.

Catroux (1991) showed that the inoculants had minimum standard of 10^6 rhizobial cell per seed for soybean.

Thompson (1991) was studied steps in the production of legume inoculants and reported massive growth of a selective rhizobal strain in liquid medium.

Jacob and Durgue (1992) inoculated eight soybean cultivars with two strains of *Bradyrhizobium japonicum* resulted in highest number of pods, seed plant and seed yield, nodule weight 100 seed weight and N fixation in IAC-2 but plant height, stem diameter and number of branches were highest in IP-240-663.

Katiyar and Pant (1993) revealed that soybean seed treatment with *Bradyrhizobium* inoculant slurry in 10 per cent gum arabic gave highest nodule mass (25.5 mg/pl.), plant dry weight (4.2 g/pl), plant nitrogen (2.2 %) whereas number of nodules (5.8 nodules/pl) and 1000 grain weight (144.5 g) was highest when inoculants granules were applied in soil.

Ernesto and Don's (1994) modified yeast extract manitol (YEM) broth, a widely used laboratory medium for the cultivation of rhizobia, to reduce its cost and made it suitable for medium scale production of rhizobial legume.

Aslam *et al.* (1995) studied importance of soybean and nutrient status of soybean crop in human diet.

Hynes *et al.* (1995) showed that the performance of liquid rhizobial formulations compared to that of peat-based products under field conditions.

Prabhakaran and Ravi (1996) showed that rhizobial bioinoculation given to soybean (*Glycine max* L.) had registered a

significant enhancement in the plant biomass (1.99 g/pl), root nodulation (25/pl), nodule biomass (87 mg/pl), shoot length (34.8 cm/pl), root length (15.6 cm/pl), total dry matter production (44.0 q/ha), grain yield (995 kg/ha) and harvest index (22.6) over the untreated control.

Thakare *et al.* (1996) observed that inoculation of soybean with *Rhizobium* produced maximum number of nodules, dry weight of nodules and shoot and grain yield over uninoculated control.

More and Jadhav (1997) found *Rhizobium* inoculation more effective in enhancing grain yield, nodule number and volume and N-content (%) in plant in soybean crop.

Pramila Rani and Kodandaramaiah (1997) observed substantial increase in yield of soybean due to *Rhizobium* treatment over no inoculation, due to increase in number of nodules/plant and nodule dry weight, better development and high dry matter accumulation, more pods/plant and plant height.

Lupwayi *et al.* (2000) showed the most important factor for inoculants quality was a high number of live rhizobia and no or minimal contamination by micro-organisms.

Aouani *et al.* (2001) Studied the symbiotic role of *Rhizobium* in fixing the atmospheric Nitrogen with leguminous crop.

Ghandour *et al.* (2001) showed that when mixture of liquid and peat based inoculants were applied, better result in

dry matter grain yield and total 'N' uptake were obtained over individual biofertilizers in Faba bean.

Maurice *et al.* (2001) studied several liquid formulations available today to sustain high viable rhizobial numbers for extended periods of time, and physiological changes in aspects such as on seed stability and their ability to form nodules in *Rhizobium* that had been stored in commercial liquid formulation for several years.

Hegde (2002) studied National level on farm trial liquid *Rhizobium* inoculants of Kharif Soybean, Pigeon pea and Groundnut.

Parr *et al.* (2002) studied effect of biofertilizers on soil ecosystem and plant growth.

Chandra *et al.* (2005) showed that *Azotobacter*, KMB, *Rhizobium* maintain shelf life upto 6 months in solid material except PSM which survives upto 8 months. But in case of liquid formulations of *Azotobacter*, PSM and KMB survives upto 2 years followed by *Rhizobium* only 14 months. This shows superiority of liquid formulation over carrier based formulations.

Panlada (2005) developed the formulation of rhizobial liquid inoculants compared with several components such as culture media, carriers and rhizobia. He also studied the cost of production, cost of materials and found that cassava produced the highest amount of reducing sugar after fermentation with fungus (*Chlamydomucor suti*). In liquid inoculants production, six different carriers were investigated with various strain of

rhizobia also found that rhizobia survival in carriers after applied to seed less than 40°C.

Rudresh *et al.* (2005) had been reported that inoculation of seed with *Rhizobium* enhanced nodulation, growth and yield response of legumes.

Singaravel *et al.* (2008) found that application of liquid biofertilizers significantly increase the growth characters like height of plant, number of branches, dry matter and yield of okra.

Chain (2010) reported that by applying appropriate liquid biofertilizers, the overall cost of production could be lower and would improve the soil quality and yield as compared to traditional chemical fertilizers.

Mane (2011) indicated the beneficial effect of liquid *Azotobacter* culture on the growth of maize crops than lignite based *Azotobacter* culture.

Pawar *et al.* (2013) a case study of soybean crop production, installed capacity and utilized capacity of oil plants in Maharashtra, India.

Tagore *et al.* (2013) reported effect of *Rhizobium* and PSB trait on nodule formation leghaemoglobin content and yield of chickpea.

2.2 Effect of seed inoculation with PSB on growth and yield of soybean and other crop

Pikovaskaya (1949) for the first time isolated an organism capable of actively decomposing tricalcium phosphate

with formation of water soluble phosphorus from soil and phosphate. The organisms was named as “Bacterium P”. Bacterium P was regarded as a promising new bacterial fertilizer in those day.

Kushadev (1956) studied the effect of phsophobacterin on yield and protein content in grains of autumn sown wheat, maize and soybean and reported that inoculation of seed with liquid phosphobacerin @ 50 ml ha⁻¹ marketadly increased the yield and protein content in grains.

Taha *et al.* (1969) treated phosphate dissolving bacteria to barley seeds which enhanced the plant growth and increased the phosphorus uptake.

Wani *et al.* (1980) recorded the phosphate solubilizing activity of four phosphomicrobials viz., *Bacillus polymyxa*, *Pseudomonas striata*, *Penicillium digitatum*, *Aspergillus awamori*. All these isolates were more efficiently in solubilizing tricalcium phosphate than rock phosphate in liquid medium.

Li (1981) studied in pot test inoculated with *Bacillus* strains and showed that the growth of soybean increased significantly.

Garside and Fulton (1986) observed that increase in phosphorus application increased the protein content of soybean.

Beheray and Agate (1989) reported that the inoculation with phosphate solubilizing microorganisms

increased the dry weight and green weight of shoot of cowpea plants.

Gaur (1989) reported that addition of phosphate to soil inoculation with phosphate solubilizing microorganisms was found to make P available to plant in netural to alkalie soil significant yield increased by application of rock phosphate and many phosphate solubilizing microorganisms have been reported in wheat, paddy and soybean.

Gaur (1990) reviewed that the significant increase in phosphorus uptake by grain was due to phosphate solubilizers inoculation both in treatments (without dry phosphatic fertilizers and those having rock phosphate treatments) and nitrogen uptake was also favourbly influenced due to greater phosphorus availability to crop.

Jayapaul and Ganesharaja (1990) reported that increase in the levels of phosphorus from 40 to 120 kg P_2O_5 ha⁻¹ significantly increased the protein content.

Dileep Kumar and Dube (1992) observed that bacterization of soybean and chickpea seeds with a siderophore producing fluorescent pseudomonad RBT-13 resulted in increased seed germination, growth (in terms of length and weight of root and shoot) and yield of the plants. Also reduced the number of chickpea wilted plants in wilt sick soil by 52 per cent making the organism a potential biocontrol agent against chickpea.

Illmer and Sacchinner, (1992) studied many soil microorganisms playing important role in solubilizing inorganic phosphates.

Nahas *et al.* (1994) isolated 31 phosphate solubilizing bacteria and 11 phosphate solubilizing fungi from 13 different soil types from various region of Sao Paulo State, Brazil. Total bacterial and fungal population ranged from 2.7 to 62.6 x 10⁵ and 2.3 to 57.7 x 10³ cfu g⁻¹ of soil, respectively.

Khamparia (1995) observed that plant height, number of branches per plant, number of pods per plant and total dry matter yield of soybean, groundnut and mung were increased with phosphorus application in combination with phosphate solubilizing microphos culture.

Mehta *et al.* (1995) reported that phosphatic biofertilizer culture when inoculated with seeds of groundnut significantly improved yield attributes viz., number of primary branches plant⁻¹, number of flowers, number of pods plant⁻¹ and pod weight plant⁻¹ over control.

Pant *et al.* (1995) reported that in soybean there was a significant increase in the number of nodules and nodules dry weight with dual inoculation of *Pseudomonas striata* and *B. japonicum* at the same rate of phosphorus have helped in solubilization of added phosphorus resulting in increase in nodulation. Similar trend were observed with yield attributing characters viz., number of pods, number of days to flowering, number of grains per pod and 1000 grain weight.

Yeole and Dube (1997) tested six fluorescent pseudomonads (CHR B₁, COR B₁, GNR B₁, GNR B₂, GNR B₃ and SBR B₅) for their effect on growth and yield of chilli, cotton, groundnut and soybean through seed bacterization. The seed germination of all crops increased by 12 to 20 per cent. Similar significant rise was noted in shoot height, root length, fresh weight, dry weight and yield.

Kim *et al.* (1998) observed that population of PSB depends on different soil properties and cultural activities.

Cattelan *et al.* (1999) examined 116 isolates from bulk soil and the rhizosphere of soybean [*Glycine max* (L.) Merr] and observed that inoculation of *Pseudomonas fluorescens*, *P. savastanoi*, *P. cepacia*, *P. putida*, *Bacillus megaterium*, and certain other unknown rhizobacteria significantly increased shoot height, shoot dry weight, root length, root dry weight, nodule number and dry weight of nodule of soybean due to their plant growth promoting activities.

Rodriguez and Fraga (1999) reported the use of PSB as inoculants increase P uptake by the plant and crop yields.

Vazquez *et al.* (2000) reported that the four *Bacillus* strains have phosphate solubilizing ability of 20 to 110 mg/L.

Singh *et al.* (2005) reported that, phosphorus is an essential nutrient for pulse crop as it helps in improving nitrogen fixation plant growth and grain yield. PSB are known to solubilize the fixed forms of phosphorus in soil and make

available to the plants. PSB inoculation increased the grain yield over uninoculated control.

Nikolay Vassilev (2006) reported microbial mediated solubilisation of insoluble phosphates through release of organic acids.

Swarnalakshmi and Mohansingh (2006) reported that *Rhizobium* inoculation of chickpea seed increased nodulation, nodule weight, shoot weight and N content in shoot and also increase *Rhizobium* PSB count.

Gupta *et al.* (2012) isolation and identification of liquid PSB able to enhance the growth and aloin-A biosynthesis of *Aloe barbadernis* miller.

2.3 Combined effect of *Rhizobium* and PSB liquid biofertilizer on growth and yield of soybean and other crop

Smith and Miller (1974) also studied the effect of nine rhizosphere bacterial inoculation on nodulation of soybean. Although eight of the rhizosphere isolates inhibited *B. japonicum* on agar. These inhibitory effect of *Pseudomonas* spp. on *Bradyrhizobium in vitro* have been shown to be caused by siderophore induced iron deprivation.

Natarajan *et al.* (1980) reported that French beans and peas treated with combined inoculation of *Rhizobium* and phosphobacteria had significant increase in yield especially in case of seeds treated with *Rhizobium* + phosphobacteria in combination over their individual treatments.

Dudeja *et al.* (1981) found that *Rhizobium* and PSMs viz., *A. awamorii* and *Pseudomonas striata* when used as seed inoculants, increased grain yield of chickpea under field conditions. Similar results were obtained from French bean (*Phaseolus vulgaris*) when inoculated with *Agrobacterium*, a phosphate solubilizer.

Kaucey (1987) reported that beans grown in autoclaved soil and inoculated with *Penicillium bilax* another phosphate solubilizer and *Rhizobium* in *Phaseolus* showed no significant increase in dry matter and total P uptake.

Alagawadi and Gaur (1988) studied associative effect of *Rhizobium* and phosphate solubilizing bacteria on yield and nutrient uptake of chickpea.

Nishijima *et al.* (1988) showed that *Pseudomonas fluorescens* SSJ-2 enhanced the nodulation ability of two strains of *Bradyrhizobium japonicum* USDA-1-110 and 61A76. Dual inoculation of *B. japonicum* with *Pseudomonas fluorescens* SS-J2 enhanced the nodulation ability. Preincubation of *B. japonicum* with *Pseudomonas fluorescens* further increased the level of nodulation indicating a beneficial interaction. Thus, *Pseudomonas* may be enhancing the nodulation of *Bradyrhizobium* by influencing the bacteria rather than plant itself.

Agarwal and Tilak (1989) found that seed inoculation with *Azospirillum brasilense* strain 4 and *Rhizobium* sp. for chickpea (*Cicer arietinum*) and cowpea (*Vigna unguiculata*)

increased the dry matter content of shoots and nitrogen uptake in plants of minor millets.

Kawale *et al.* (1989) reported significant increase in dry weight of leaves, shoot and root with dual inoculation (phosphate solubilizing bacteria and *Rhizobium*) in soybean over inoculation with *Rhizobium* alone.

Singh and Gaur (1992) studied the influence of rhizospheric bacteria on a competitive ability of introduced strain and proved the nodulation as well as competitiveness of an effective strain of chickpea *Rhizobium*.

Shabdeev *et al.* (1992) studied the effect of dual inoculation of soybean seeds with *Bradyrhizobium japonicum* and *P. fluorescens*. They reported that dual inoculation enhanced seed yield.

Narayanan (1994) showed that combination with phosphobacteria and slow growing *Rhizobium* strain MTP recorded significant increase in plant growth, nodulation and seed yield over the individual inoculation.

Saxena and Tilak (1994) studied beneficial interaction among N fixers, phosphate solubilizing and nitrogen fixing micro organisms, vesicular arbuscular mycorrhizae with N₂ fixers, VAM fungi with phosphate solubilizing microorganisms, plant growth promoting bacteria and *Rhizobium*.

Natarajan and Subramanian (1995) showed that *Rhizobium* strain T₉ along with phosphobacteria 75 per cent phosphorus level recorded higher nodule number, shoot length,

root length and increased pod yield than the dual inoculation at 50 per cent P level in groundnut.

Srivastava and Ahlawat (1995) reported that peas cv. Arkel inoculated with *Rhizobium* and PSB had increased nodulation, plant height, dry matter accumulation, number of pods/plant, seeds/pod, seed yield and N and P uptake increased with increasing P rate, increasing growth, yield components and seed yield.

Prabhakaran *et al.* (1996) recommended seed bacterization of Vamban-1 blackgram with *Rhizobium* and *B. megaterium* for increased grain yield and they found that inoculation increased the grain yield by 37.2 per cent over untreated control.

Shivkumar (1998) conducted a field trial on chickpea and gave the information on yield correlations from the data on the number of seeds per pod and pods per plant as well as the seed and straw yields. The yield obtained with this treatment was higher with that produced by *Rhizobium* inoculation, but was at par with the yield obtained with PSB inoculation.

Bhattacharya and Kumar (2000) states significance of liquid biofertilizer over carrier based biofertilizer, i.e. high microbial population density, longer self life.

Vijila and Jebaraj (2003) conducted field study in Vamban, Tamil Nadu in two nutrients acid-stress soil, even strains of *Rhizobium* were compared for their symbiotic association with the host legume green gram. The acid tolerant

strain demonstrated a comparative advantage over the acid sensitive strains in their ability to nodulate and thereby promoting the growth and yield of green gram. The combined inoculation of this strain with PSB improved yield of crop.

Tyagi *et al.* (2004) reported that pea (*Pisum sativum* L.) treated with *Rhizobium* and phosphate solubilizing bacteria and observed that among various biofertilizers treatments, the maximum grain and straw yields were recorded with the combined use of *Rhizobium* + PSB and mixed culture treatments over their individual treatments.

Tran Thi Ngoc Son *et al.* (2007) effect of co-inoculation of (*Bradyrhizobia* and phosphate solubilizing bacteria) liquid on soybean under rice based cropping system.

Aftab Afzal and Asghari Bano Mussaratfatima (2009) demonstrated that higher soybean yield by inoculation with both N fixing and P-solubilizing bacteria.

Pindi and Satyanarayana (2012) stated significant use of liquid biofertilizers over carrier based biofertilizers.

3. MATERIAL AND METHODS

The present investigation entitled, “Studies on seed inoculation of MPKV’s *Rhizobium* and PSB Liquid Biofertilizer in Soybean.” was carried out at the farm area of Department of Plant Pathology and Agricultural Microbiology, Post Graduate Institute, M.P.K.V., Rahuri and *in vitro* studies were carried out in laboratory, Department of Plant Pathology and Agricultural Microbiology, Post Graduate Institute, M.P.K.V., Rahuri. The details of material required and methods followed during the period of investigation are described briefly in the succeeding paragraphs.

3.1 Material

3.1.1 Seeds

The seed of Soybean variety JS-335 required for the field experiment were obtained from pulse improvement project Mahatma Phule Krishi Vidyapeeth Rahuri.

3.1.2 Field

The experiment was conducted at the farm area of Department of Plant Pathology and Agricultural Microbiology, Post Graduate Institute, M.P.K.V., Rahuri .

3.1.3 Collection of soil sample

As per the treatments soil samples were collected from each replication from experimental plot and representative sample was used for microbial and chemical analysis.

3.1.4 *Rhizobium* liquid Biofertilizer

The *Rhizobium* liquid Biofertilizer was obtained from Biofertilizer Production Unit, Department of Plant Pathology and Agricultural Microbiology, M.P.K.V., Rahuri.

3.1.5 PSB Liquid Biofertilizer

The PSB liquid Biofertilizer was obtained from Biofertilizer Production Unit, Department of Plant Pathology and Agricultural Microbiology, M.P.K.V., Rahuri.

3.1.6 Fertilizers

The basal dose of Nitrogen supplied through urea @ 50 kg N/ha, while that of Phosphorus through the single superphosphate @ 75 kg P₂O₅/ha.

3.1.7 Insecticides and Fungicides

Seed treatment with carbendazim @ 2.5 gm/kg seed for the protection from fungal disease.

3.1.8 Microbial analysis of soil

1. Soil

The representative soil samples were collected from root zone of Soybean crop which was treated with different biofertilizer treatments in three replications.

2. Culture media

Various culture media were used for estimation of microbial count i.e. *Rhizobium* and phosphate solubilizing bacteria during the experiment. The culture media and their composition are given below.

a. Congo Red Yeast Extract Mannitol Agar Medium (CRYEMA)

- | | | | |
|-----|---|---|---------|
| 1. | Mannitol | : | 10.0 gm |
| 2. | KH_2PO_4 | : | 0.5 gm |
| 3. | $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ | : | 0.2 gm |
| 4. | NaCl | : | 0.1 gm |
| 5. | CaCO_3 | : | 3.0 gm |
| 6. | Congo red (1%) | : | 2.5 ml |
| 7. | Yeast extract | : | 1 gm |
| 8. | Agar Agar | : | 20 gm |
| 9. | Distilled water | : | 1000 ml |
| 10. | pH | : | 7 |

b. Pikovaskaya's media for Phosphate solubilizing microorganism

- | | | | |
|-----|-------------------------------------|---|-----------|
| 1. | Glucose | : | 10 g |
| 2. | $\text{Ca}_3(\text{PO}_4)_2$ | : | 5 g |
| 3. | $(\text{NH}_4)_2\text{SO}_4$ | : | 0.5 g |
| 4. | KCl | : | 0.2 g |
| 5. | $\text{MgSO}_4, \text{H}_2\text{O}$ | : | 0.1 g |
| 6. | MnSO_4 | : | Trace |
| 7. | FeSO_4 | : | Trace |
| 8. | Yeast extract | : | 0.5 g |
| 9. | Agar | : | 15 g |
| 10. | Distilled water | : | 1000 ml |
| 11. | pH | : | 7.0 - 7.2 |

3.1.9 Glass wares

The Corning brand glass wares *viz.*, beakers, pipettes, test tubes, conical flasks, volumetric flasks, funnels, measuring cylinders, microscopic glass slides, cover slips, petridishes, oven dry etc. were used whenever necessary.

3.1.10 Equipments

The laboratory equipments *viz.*, autoclave, incubator, laminar air flow cabinet, research microscope, colony counter refrigerator, weighing balance, grinding machine, hot air oven, test tube shaker, etc.

3.1.11 Miscellaneous material

Labels, knives, polythene bags, rubber bands, inoculating needle, spirit lamp, thread, used in laboratory whenever necessary.

3.2 Methods

3.2.1 Field experiment

A field experiment was conducted to study the effect of seed inoculation of MPKV'S liquid *Rhizobium* and PSB liquid biofertilizer on growth and yield of soybean crop.

3.2.2 Experimental Details

- | | | | |
|----|--------------|---|--|
| 1. | Design | : | RBD for field |
| 2. | Treatments | : | 8 |
| 3. | Replications | : | 3 |
| 4. | Season | : | Kharif -2013 |
| 5. | Variety | : | JS-335 |
| 6. | Spacing | : | 30 x 10 cm |
| 7. | NPK dose | : | 50 kg N and 75 P ₂ O ₅ |
| 8. | Seed rate | : | 75 kg /ha |

Treatment details

- T₁ : Seed inoculation of *Rhizobium* liquid biofertilizer @ 10 ml/kg.
- T₂ : Seed inoculation of *Rhizobium* liquid biofertilizer @ 25 ml / kg.
- T₃ : Seed inoculation of PSB liquid biofertilizer @ 10 ml/kg.
- T₄ : Seed inoculation of PSB liquid biofertilizer @ 25 ml/kg.
- T₅ : Seed inoculation of *Rhizobium* liquid biofertilizer + PSB Liquid biofertilizer @ 10 ml each/kg.
- T₆ : Seed inoculation of *Rhizobium* liquid biofertilizer + PSB Liquid biofertilizer @ 25 ml each/kg.
- T₇ : Seed inoculation with MPKV's standard dose of lignite based *Rhizobium* + PSB @ 25 g each/kg of seed
- T₈ : Control.

3.2.3 Determination of *Rhizobium* population from soil

The soil samples collected from soybean plot of different treatments were subjected to microbiological analysis for determination of viable count of *Rhizobium* by serial dilution and pour plate technique on CRYEMA nutrient media, as follows:

1. One gram soil sample was added in the 9 ml sterile water in test tube. Test tube was shaken thoroughly for 1 minute to get 10⁻¹ dilution.
2. One ml suspension of 10⁻¹ dilution was transferred to next 9 ml sterile water blank and mixed thoroughly by shaking for one min. This gave 10⁻² dilution.

3. In similar way 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , 10^{-7} and 10^{-8} dilutions of the soil sample in test tube containing sterile water were obtained.
4. The dilution 10^{-5} to 10^{-7} were used for estimation of *Rhizobium* count. All the petriplates were labeled properly.
5. One ml aliquot of desired dilution was transferred to sterile petriplates and poured with the required molten medium for each organism.
6. The soil dilution was mixed with the molten medium by gentle rotating and all plates were allowed to solidify the medium.
7. The Petriplates were incubated at $28-30^{\circ}\text{C}$ for a week and observations were recorded.

The microbial count was calculated by multiplying the average plate count with the dilution factor (Csppucino and Sherman, 1987).

$$\text{No. of microbes / g} = \frac{\text{Average plate count} \times \text{Dilution factor}}{\text{Oven dry soil} \quad \text{Oven dry weight of 1 g soil sample}}$$

3.2.4 Determination of PSB Population from soil

The soil samples collected from soybean plot of different treatments were subjected to microbiological analysis for determination of viable count of *PSB* by serial dilution and pour plate technique (Pikovaskaya, 1948) on Pikovaskaya's nutrient media, as follows,

1. One g soil sample was added in the 9 ml sterile water in test tube. Test tube was shaken thoroughly for 1 minute to get 10^{-1} dilution.
2. One ml suspension of 10^{-1} dilution was transferred to next 9 ml sterile water blank and mixed thoroughly by shaking for one min. This gave 10^{-2} dilution.
3. In similar way 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , 10^{-7} and 10^{-8} dilutions of the soil sample in test tube containing sterile water were obtained.
4. The dilution 10^{-5} to 10^{-7} were used for estimation of PSB count. All the petriplates were labeled properly.
5. One ml aliquot of desired dilution was transferred to sterile petriplates and poured with the required molten medium for each organism.
6. The soil dilution was mixed with the molten medium by gentle rotating and all plates were allowed to solidify the medium.
7. The Petriplates were incubated at $28-30^{\circ}\text{C}$ for a week and observations were recorded. The microbial colonies showing the transparent clear zones around their colony were selected and count was recorded (Rao and Sinha, 1963).

The microbial count was calculated by multiplying the average plate count with the dilution factor (Cuppucino and Sherman, 1987).

$$\text{No. of microbes / g} = \frac{\text{Average plate count X Dilution factor}}{\text{Oven dry weight of 1 g soil sample}}$$

Oven dry soil

3.2.5 Determination of leghaemoglobin content in fresh soybean root nodule

The leghaemoglobin content (optical density /gm)of nodule at 60 days of soybean plant growth was estimated by following procedure :

1. Nodule from freshly uprooted plants of soybean were removed and washed with clean water to remove dirt and dust particles sticking to the nodules.
2. Nodules then kept on blotting paper to remove the excess of water.
3. These fresh nodules were then weighted to exact 1 gm on chemical balance.
4. They were then transferred to a clean mortar .Two spoonful of sodium hyposulphite was added to the mortar containing a nodules ,with the help of spatula.
5. The nodule were properly crushed with sodium hyposulphide to fine paste.
6. To this 4 ml fresh cold pyridine was added in which leghaemoglobin was dissolved this paste was transferred to the centrifugal tube
7. Again 4 ml fresh cold pyridine were added to the mortar to wash the mortar and pestle and that washing were transferred to the same centrifugal tube.

8. Then, 2ml pyridine were added to the centrifugal tube to make the exact volume of total solution.
9. The final volume made to 10 ml and centrifuged at 2000 rpm for 30 minutes to throw down any contaminated particles. And the clear supernatant was collected.
10. The optical density of leghaemoglobin measured on spectronic -20 at 550 nm wave length as OD/gm of nodule.

3.2.6 Observations recorded

A. Microbial observations

1. To record initial microbial population before application of liquid biofertilizers.
2. To record the microbial population after 30, 60, and 90 days after application of liquid biofertilizers.

B. Effect of liquid biofertilizer On different growth parameters

1. Nodulation pattern studies at 30, 60, 90 days after of sowing
2. Nodulation pattern : white, pink and black nodules
3. Nodulation pattern : main root and sub root.
4. Leghaemoglobin content in nodule
5. Plant height at 30, 60 and 90 days
6. Days to 50 per cent flowering
7. No of pods per plant
8. 1000 grain weight
9. Yield

3.2.7 Statistical analysis

The data were subjected to statistical analysis by Micro- stat method (Panse and Sunkhatme, 1957).

4. RESULTS

A field experiment entitled “Studies on seed inoculation of MPKV’s *Rhizobium* and PSB liquid biofertilizer in soybean was conducted during *kharif* 2013. The results of experiment are presented in this chapter.

4.1 Lab analysis

***Rhizobium* count at 10^5 (cfu g⁻¹ soil) at 30, 60, 90 DAS**

The results in respect of *Rhizobium* count at 30 days after sowing presented in (Table 1) were significant for the different treatments given to soybean seed.

All the inoculation treatments recorded significantly superior *Rhizobium* count than uninoculation (control) treatments. Treatment T₆ was recorded significantly highest *Rhizobium* count (32×10^5 cfu g⁻¹ soil) followed by T₂ with rhizobium count (30×10^5 cfu g⁻¹ soil) which were at par with each other, T₈ (Control/uninoculated seed) recorded the least *Rhizobium* count (7×10^5 cfu g⁻¹ soil).

The results in general, indicated that the inoculation of soybean seed with liquid *Rhizobium* biofertilizer treatments recorded significantly more count and amongst them the combined liquid inoculation treatment (i.e. treatment T₆, 25 ml *Rhizobium* + 25 ml PSB kg⁻¹ of seed) recorded maximum *Rhizobium* count than seed inoculation with lignite base

Table 1. *Rhizobium* count at 30, 60, 90 DAS at 10^5 (cfu g⁻¹ soil) as influenced by various treatments in soybean

Treatment	30 DAS	60 DAS	90 DAS
T ₁ : Liquid <i>Rhizobium</i> 10 ml kg ⁻¹ of seed	20.00 (4.47)	28.00 (5.29)	21.00 (4.55)
T ₂ : Liquid <i>Rhizobium</i> 25 ml kg ⁻¹ of seed	30.00 (5.48)	35.00 (5.92)	29.00 (5.39)
T ₃ : Liquid PSB 10 ml kg ⁻¹ of seed	15.00 (3.87)	16.00 (4.06)	14.00 (3.74)
T ₄ : Liquid PSB 25 ml kg ⁻¹ of seed	17.00 (4.12)	19.00 (4.36)	16.00 (4.00)
T ₅ : Liquid <i>Rhizobium</i> + PSB @ 10 ml each kg ⁻¹ of seed	26.00 (5.09)	33.00 (5.74)	25.00 (5.00)
T ₆ : Liquid <i>Rhizobium</i> + PSB @ 25 ml each kg ⁻¹ of seed	32.00 (5.66)	38.00 (6.16)	33.00 (5.74)
T ₇ : Lignite based <i>Rhizobium</i> + PSB @ 25 g each kg ⁻¹ of seed	19.00 (4.36)	27.00 (5.20)	18.00 (4.24)
T ₈ : Control	7.00 (2.65)	9.00 (3.00)	8.00 (2.82)
S.E. $\pm$	1.24	2.34	2.27
CD at 5 %	3.72	7.11	6.89

Initial rhizobial population – 5×10^5 cfu g⁻¹ soil

Figures in parenthesis indicates the square root values

biofertilizer i.e. T₇ (25 g *Rhizobium* + 25 g PSB kg⁻¹ of seed) with *rhizobium* count (19 x 10⁵ cfu g⁻¹ soil).

The results recorded at 60 DAS were presented in (Table 1) on *Rhizobium* count were significant for all treatments. Treatment T₆ recorded significantly highest *Rhizobium* count i.e. (38 x 10⁵ cfu g⁻¹ soil), Followed by T₂ with *Rhizobium* count (35 x 10⁵ cfu g⁻¹ soil) which were at par with each other . T₈ (Control/Uninoculated) was recorded with least *Rhizobium* count i.e. (9 x 10⁵ cfu g⁻¹ soil).

The results in general, indicated that the inoculation of soybean seed with liquid biofertilizer treatments recorded significantly more count and amongst them the combined liquid inoculation treatment T₆ (25 ml *Rhizobium* + 25 ml PSB kg⁻¹ of seed) recorded maximum *Rhizobium* count (38 cfu x 10⁵ g⁻¹ soil) than inoculation with MPKV'S lignite base biofertilizer Treatment i.e T₇ (25 gm *Rhizobium* + 25 g PSB kg⁻¹ of seed) with *Rhizobium* count (27 cfu x 10⁵ g⁻¹ soil).

The results indicated that the number of *Rhizobium* counts were increased at 60 days after sowing as compared to 30 days after sowing which reflects high *Rhizobium* microbial activity at 60 days after sowing.

The data recorded at 90 DAS presented in (Table-1) on *Rhizobium* count were significant for all the treatments. Treatment T₆ recorded significantly highest *Rhizobium* count i.e. (33 x 10⁵ cfu g⁻¹ soil) followed by T₂ with *Rhizobium* count (29 x 10⁵ cfu g⁻¹ soil), T₅ with *Rhizobium* count (25 x 10⁵ cfu g⁻¹ soil).

T₈ (Control) was recorded with least *Rhizobium* count i.e. (8×10^5 cfu g⁻¹ soil).

The results in general recorded that the inoculation of seed with liquid *Rhizobium* biofertilizer treatments recorded significantly more count and amongst them the combined effect of liquid *Rhizobium* and PSB inoculation treatment i.e. T₆ (25 ml *Rhizobium* + 25 ml PSB kg⁻¹ seed) and T₅ were significantly superior over lignite base inoculation i.e. T₇ (25 g *Rhizobium* + 25 g PSB kg⁻¹ of seed) with *Rhizobium* count (18×10^5 cfu g⁻¹ soil).

The results clearly indicated that the number of *Rhizobium* counts were decreased at 90 days after sowing as compared with *Rhizobium* count at 60 DAS which reflected that the activity of *Rhizobium* microbial activities declined gradually after active growth period i.e. (60 DAS).

4.2 PSB count at 10^5 (cfu g⁻¹ soil) at 30, 60, 90 DAS

The results in respect of PSB count at 30 DAS presented in (Table 2) were significant for all the treatments. Among all the treatments T₆ recorded highest PSB count i.e. (38×10^5 cfu g⁻¹ soil) followed by treatment T₄ with PSB count (36×10^5 cfu g⁻¹ soil) which were at par with each other. Treatment T₈ (Control /uninoculated) was recorded with least PSB count (10×10^5 cfu g⁻¹ soil).

The results in general indicated that the inoculation of seed with liquid PSB biofertilizer treatments recorded significantly more count and amongst them the combined effect of liquid PSB inoculation treatment i.e. T₆ (25 ml *Rhizobium* + 25

Table 2. PSB count at 30, 60 , 90 DAS at 10^5 cfu g⁻¹ soil as influenced by various treatments in soybean

Treatment	30 DAS	60 DAS	90 DAS
T ₁ : Liquid <i>Rhizobium</i> 10 ml kg ⁻¹ of seed	15.00 (3.87)	18.00 (4.24)	17.00 (4.12)
T ₂ : Liquid <i>Rhizobium</i> 25 ml kg ⁻¹ of seed	17.00 (4.12)	21.00 (5.58)	18.00 (4.29)
T ₃ : Liquid PSB 10 ml kg ⁻¹ of seed	29.00 (5.39)	33.00 (5.74)	31.00 (5.57)
T ₄ : Liquid PSB 25 ml kg ⁻¹ of seed	36.00 (6.00)	42.00 (6.48)	38.00 (6.16)
T ₅ : Liquid <i>Rhizobium</i> + PSB @ 10 ml each kg ⁻¹ of seed	33.00 (5.74)	40.00 (6.32)	33.00 (5.74)
T ₆ : Liquid <i>Rhizobium</i> + PSB @ 25 ml each kg ⁻¹ of seed	38.00 (6.16)	45.00 (6.70)	39.00 (6.24)
T ₇ : Lignite based <i>Rhizobium</i> + PSB @ 25 g each kg ⁻¹ of seed	28.00 (5.29)	32.00 (5.66)	27.00 (5.19)
T ₈ : Control	10.00 (3.16)	15.00 (3.87)	11.00 (3.31)
S.E. ±	2.25	2.29	2.64
CD at 5 %	6.82	6.94	8.01

Initial PSB population – 7×10^5 cfu g⁻¹ soil

Figures in parenthesis indicates the square root values

ml PSB kg⁻¹) and T₅ (10 ml *Rhizobium* + 10 ml PSB kg⁻¹) were significantly superior over lignite base inoculation i.e. T₇ (25 g *Rhizobium* + 25 g PSB kg⁻¹ of seed) with PSB count (28 x 10⁵ cfu g⁻¹ soil).

The data recorded at 60 DAS presented in (Table 2) were significantly superior for all the treatments. Seed inoculation with treatment T₆ recorded significantly highest PSB count (45 x 10⁵ cfu g⁻¹ soil) followed by treatment T₄ with PSB count (42 x 10⁵ cfu g⁻¹ soil), T₅ with PSB count (40 x 10⁵ cfu g⁻¹ soil). Treatment T₅ was at par with treatment T₆. Treatment T₈ (Control/uninoculated treatment) was recorded with least PSB count i.e. (15 x 10⁵ cfu g⁻¹ soil).

The results in general showed that the inoculation of seed with liquid PSB biofertilizer treatments recorded significantly more count and amongst them the combined effect of PSB *Rhizobium* liquid biofertilizer i.e. treatment T₆ (25 ml *Rhizobium* + 25 ml PSB liquid biofertilizer kg⁻¹ of seed) with PSB count (45 x 10⁵ cfu g⁻¹ soil) and T₅ (10 ml *Rhizobium* + 10 ml PSB liquid biofertilizer kg⁻¹ of seed) with PSB count (40 x 10⁵ cfu g⁻¹ soil) were significantly superior over MPKV'S lignite base seed inoculation treatment T₇ (25 g *Rhizobium* + 25 g PSB kg⁻¹ of seed) with PSB count (32 x 10⁵ cfu g⁻¹ soil).

The result clearly indicated that the PSB count increased gradually from 30 DAS which were maximum at 60 DAS. This sowed that the higher PSB microbial activity at 60 DAS.

The results in respect of 90 DAS presented in (Table 2) on PSB count were significant for all the treatments. Among all the treatments T₆ recorded highest PSB count (39×10^5 cfu g⁻¹ soil) followed by treatment T₄ with PSB count (38×10^5 cfu g⁻¹ soil). Treatment T₆ and T₄ were at par with each other. Treatment T₈ (Control uninoculated treatment) was recorded with least PSB count i.e. (11×10^5 cfu g⁻¹ soil).

The result in general indicated that the inoculation of seed with liquid PSB biofertilizer treatments recorded significantly more count and amongst them the combined effect of PSB and *Rhizobium* liquid biofertilizer treatments i.e. T₆ (25 ml *Rhizobium* + 25 ml PSB MPKV's liquid biofertilizer) with PSB count (39×10^5 cfu g⁻¹ soil) and T₅ with PSB count (33×10^5 cfu g⁻¹ soil) were significantly superior over the lignite base biofertilizer treatment i.e. T₇ (25 g *Rhizobium* + 25 g PSB MPKV's lignite based biofertilizer) with PSB count (27×10^5 cfu g⁻¹ soil).

The number of PSB count were declined at 90 DAS as compared to 60 DAS. Which reflects the less microbial activity at 90 DAS as compared to 60 DAS.

4.3 Nodulation pattern : white pink and black nodules

The data regarding pigmentation i.e. (white, pink and black) of nodules at 30, 60 and 90 days of crop growth is presented in Table 3.

The data in (Table 3) revealed that at 30 DAS highest number of white nodules (26.16) were recorded in the treatment T₆ followed by T₂ (24.10) and lowest number of white nodules

Table 3. Number of nodules plant⁻¹ : white, pink and black nodules at 30 DAS as influenced by various treatments in soybean

Treatment	Number of white nodules	Number of pink nodules	Number of black nodules	Total
T ₁ : Liquid <i>Rhizobium</i> 10 ml kg ⁻¹ of seed	22.50 (4.74)	3.17 (1.78)	-	25.67 (5.06)
T ₂ : Liquid <i>Rhizobium</i> 25 ml kg ⁻¹ of seed	24.10 (4.91)	7.32 (2.70)	-	31.42 (5.60)
T ₃ : Liquid PSB 10 ml kg ⁻¹ of seed	15.19 (3.90)	2.15 (1.47)	-	17.34 (4.16)
T ₄ : Liquid PSB 25 ml kg ⁻¹ of seed	16.33 (4.04)	3.34 (1.82)	-	19.67 (4.44)
T ₅ : Liquid <i>Rhizobium</i> + PSB @ 10 ml each kg ⁻¹ of seed	23.42 (4.83)	6.42 (2.53)	-	29.84 (5.46)
T ₆ : Liquid <i>Rhizobium</i> + PSB @ 25 ml each kg ⁻¹ of seed	26.16 (5.11)	8.56 (2.93)	-	34.72 (5.89)
T ₇ : Lignite based <i>Rhizobium</i> + PSB @ 25 g each kg ⁻¹ of seed	18.00 (4.24)	3.05 (1.75)	-	21.05 (4.59)
T ₈ : Control	11.83 (3.43)	1.38 (1.17)	-	13.21 (3.63)
S.E. $\pm$	1.82	1.17	-	1.39
CD at 5 %	5.55	3.56	-	4.25

Figures in parenthesis indicates the square root values

were recorded in the treatment T₈ (11.83) . The highest number of pink nodules (8.56) were recorded in the treatment T₆ followed by T₂ (7.32). Treatment T₂ and treatment T₆ were at par with each other. The least number of pink nodules were recorded in treatment T₈ (1.38). The lignite based treatment T₇ showed less number of both white nodules (18.00) and pink nodules (3.05) as compared to combined treatment of MPKV'S *Rhizobium* + PSB liquid biofertilizer treatment i.e. treatment T₆ and T₅.

The highest number of white nodules at 60 DAS presented in (Table 4) were recorded in treatment T₆ (4.17) and T₂ (3.43) and the lowest number recorded in the treatments T₈ (1.67). The highest number of pink nodules at 60 DAS were recorded in treatment T₆ (61.83) followed by T₂ (59.23) which were at par with each other and the lowest number recorded in treatment T₈ (20.67). The highest number of black nodules recorded at 60 DAS in treatment T₈ (6.67) the lowest number of black nodules recorded at 60 DAS in treatment T₆ (1.33).

The highest number of white nodules at 90 DAS presented in (Table 5) were recorded in treatment T₆ (0.64) followed by treatment T₁ (0.48) and the lowest number recorded in treatment T₈ (0.08). The highest number of pink nodules were recorded at 90 DAS were recorded at T₆ (9) and T₂ (8). The highest number of black nodules were recorded at T₆ (54.36) and T₂ (52.73) and lowest number of black nodules were recorded at T₈ (18.14).

Table 4. Number of nodules plant⁻¹: white, pink and black nodules at 60 DAS as influenced by various treatments in soybean

Treatment	Number of white nodules	Number of pink nodules	Number of black nodules	Total
T ₁ : Liquid <i>Rhizobium</i> 10 ml kg ⁻¹ of seed	3.00 (1.73)	48.34 (6.95)	3.00 (1.73)	54.34 (7.37)
T ₂ : Liquid <i>Rhizobium</i> 25 ml kg ⁻¹ of seed	3.43 (1.85)	59.23 (7.69)	2.00 (1.71)	64.66 (8.04)
T ₃ : Liquid PSB 10 ml kg ⁻¹ of seed	3.00 (1.73)	37.00 (6.08)	5.00 (2.23)	45.00 (6.70)
T ₄ : Liquid PSB 25 ml kg ⁻¹ of seed	3.04 (1.74)	40.34 (6.35)	4.00 (2.00)	47.38 (6.80)
T ₅ : Liquid <i>Rhizobium</i> + PSB @ 10 ml each kg ⁻¹ of seed	2.34 (1.52)	59.00 (7.68)	2.00 (1.41)	63.34 (7.95)
T ₆ : Liquid <i>Rhizobium</i> + PSB @ 25 ml each kg ⁻¹ of seed	4.17 (2.04)	61.83 (7.86)	1.33 (1.15)	67.33 (8.20)
T ₇ : Lignite based <i>Rhizobium</i> + PSB @ 25 g each kg ⁻¹ of seed	3.33 (1.82)	41.66 (6.45)	4.34 (2.08)	51.66 (7.18)
T ₈ : Control	1.67 (1.29)	20.67 (4.59)	6.67 (2.58)	26.68 (5.16)
S.E. $\pm$	1.19	2.38	1.02	1.41
CD at 5 %	3.67	7.21	3.10	4.26

Figures in parenthesis indicates the square root values

Table 5. Number of nodules plant⁻¹ : white, pink, black nodules at 90 DAS as influenced by various treatments in soybean

Treatment	Number of white nodules	Number of pink nodules	Number of black nodules	Total
T ₁ : Liquid <i>Rhizobium</i> 10 ml kg ⁻¹ of seed	0.48 (0.69)	6.50 (2.54)	46.02 (6.78)	53.00 (7.28)
T ₂ : Liquid <i>Rhizobium</i> 25 ml kg ⁻¹ of seed	0.61 (0.78)	8.00 (2.82)	52.73 (7.26)	61.34 (7.83)
T ₃ : Liquid PSB 10 ml kg ⁻¹ of seed	0.35 (0.59)	7.00 (2.64)	33.14 (5.76)	40.49 (6.36)
T ₄ : Liquid PSB 25 ml kg ⁻¹ of seed	0.41 (0.64)	4.00 (2.00)	41.60 (6.45)	46.01 (6.78)
T ₅ : Liquid <i>Rhizobium</i> + PSB @ 10 ml each kg ⁻¹ of seed	0.59 (0.76)	7.00 (8.64)	49.07 (7.00)	56.66 (7.52)
T ₆ : Liquid <i>Rhizobium</i> + PSB @ 25 ml each kg ⁻¹ of seed	0.64 (0.80)	9.00 (3.00)	54.36 (7.37)	64.00 (8.00)
T ₇ : Lignite based <i>Rhizobium</i> + PSB @ 25 g each kg ⁻¹ of seed	0.43 (0.65)	5.00 (2.23)	45.23 (6.72)	50.66 (7.12)
T ₈ : Control	0.08 (0.28)	2.00 (1.41)	18.14 (4.25)	20.22 (4.50)
S.E. $\pm$	0.03	0.98	1.79	1.78
CD at 5 %	0.09	2.94	5.43	5.41

Figures in parenthesis indicates the square root values

4.4 Nodulation pattern : main root and sub root

a. Nodulation pattern : main root and sub root at 30 DAS

The data regarding number of nodulations were revealed in (Table 6) the highest number of nodules on main root per plant were recorded in treatment T₆ (11.61) followed by T₂ (10.18), which were at par with each other. While minimum number of nodules on main root per plant were recorded in treatment T₈ (4.09).

The highest number of nodules on sub root per plant were recorded in treatment T₆ (23.11) followed by T₂ (21.24), T₅ (20.68) were at par with each other while minimum number of nodules on sub root per plant were recorded in treatment T₈ (9.12).

b. Nodulation pattern : main root and sub root at 60 DAS

The highest number of nodules (Table 7) on main root per plant were recorded in treatment T₆ (27.33) followed by T₂ (24.03) and T₅ (24.00) which were at par with each other. The lowest number of nodules were recorded in treatment T₈ (11.00).

The highest number of nodules on sub root per plant were recorded in treatment T₆ (40.00), followed by T₂ (39.62) , T₅ (39.34). both T₂ and T₅ are at par. The lowest number of nodules on sub root per plant were recorded in T₈ (15.68).

c. Nodulation pattern : main root and sub root at 90 DAS

The highest number of nodules (Table 8) on main root per plant were recorded in treatment T₆ (26.45) followed by

Table 6. Number of nodules plant⁻¹ on main root and sub root at 30 DAS as influenced by various treatments in soybean

Treatment	Number of nodules on main root	Number of nodules on sub root	Total
T ₁ : Liquid <i>Rhizobium</i> 10 ml kg ⁻¹ of seed	9.03 (3.00)	16.64 (4.07)	25.67 (5.06)
T ₂ : Liquid <i>Rhizobium</i> 25 ml kg ⁻¹ of seed	10.18 (3.19)	21.24 (4.60)	31.42 (5.60)
T ₃ : Liquid PSB 10 ml kg ⁻¹ of seed	6.19 (2.48)	11.15 (3.35)	17.34 (4.16)
T ₄ : Liquid PSB 25 ml kg ⁻¹ of seed	7.36 (2.71)	12.33 (3.51)	19.67 (4.44)
T ₅ : Liquid <i>Rhizobium</i> + PSB @ 10 ml each kg ⁻¹ of seed	9.20 (3.03)	20.68 (4.55)	29.84 (5.46)
T ₆ : Liquid <i>Rhizobium</i> + PSB @ 25 ml each kg ⁻¹ of seed	11.61 (3.40)	23.11 (4.80)	34.72 (5.89)
T ₇ : Lignite based <i>Rhizobium</i> + PSB @ 25 g each kg ⁻¹ of seed	7.04 (2.65)	14.01 (3.74)	21.05 (4.59)
T ₈ : Control	4.09 (2.02)	9.12 (3.01)	13.21 (3.63)
S.E. $\pm$	0.90	1.29	1.39
CD at 5 %	2.73	3.93	4.25

Figures in parenthesis indicates the square root values

Table 7. Number of nodules plant⁻¹ on main root and sub root at 60 DAS as influenced by various treatments in soybean

Treatment	Number of nodules on main root	Number of nodules on sub root	Total
T ₁ : Liquid <i>Rhizobium</i> 10 ml kg ⁻¹ of seed	17.00 (4.12)	37.34 (6.11)	54.34 (7.37)
T ₂ : Liquid <i>Rhizobium</i> 25 ml kg ⁻¹ of seed	24.03 (4.90)	39.62 (6.29)	64.66 (8.04)
T ₃ : Liquid PSB 10 ml kg ⁻¹ of seed	15.34 (3.91)	29.66 (5.44)	45.00 (6.70)
T ₄ : Liquid PSB 25 ml kg ⁻¹ of seed	11.38 (3.37)	36.00 (6.00)	47.38 (6.80)
T ₅ : Liquid <i>Rhizobium</i> + PSB @ 10 ml each kg ⁻¹ of seed	24.00 (4.89)	39.34 (6.21)	63.34 (7.95)
T ₆ : Liquid <i>Rhizobium</i> + PSB @ 25 ml each kg ⁻¹ of seed	27.33 (5.23)	40.00 (6.32)	67.33 (8.20)
T ₇ : Lignite based <i>Rhizobium</i> + PSB @ 25 g kg ⁻¹ of seed	19.32 (4.39)	32.34 (5.68)	51.66 (7.18)
T ₈ : Control	11.00 (3.31)	15.68 (3.96)	26.68 (5.16)
S.E. $\pm$	1.39	1.78	1.41
CD at 5 %	4.17	5.39	4.26

Figures in parenthesis indicates the square root values

Table 8. Number of nodules plant⁻¹ on main root and sub root at 90 DAS as influenced by various treatments in soybean

Treatment	Number of nodules on main root	Number of nodules on sub root	Total
T ₁ : Liquid <i>Rhizobium</i> 10 ml kg ⁻¹ of seed	19.00 (4.36)	34.00 (5.83)	53.00 (7.28)
T ₂ : Liquid <i>Rhizobium</i> 25 ml kg ⁻¹ of seed	24.12 (4.91)	37.22 (6.10)	61.34 (7.83)
T ₃ : Liquid PSB 10 ml kg ⁻¹ of seed	12.09 (3.48)	28.40 (5.32)	40.49 (6.36)
T ₄ : Liquid PSB 25 ml kg ⁻¹ of seed	13.67 (3.70)	32.34 (5.69)	46.01 (6.78)
T ₅ : Liquid <i>Rhizobium</i> + PSB @ 10 ml each kg ⁻¹ of seed	22.20 (4.71)	34.46 (5.87)	56.66 (7.52)
T ₆ : Liquid <i>Rhizobium</i> + PSB @ 25 ml each kg ⁻¹ of seed	26.45 (5.14)	37.55 (6.12)	64.00 (8.00)
T ₇ : Lignite based <i>Rhizobium</i> + PSB @ 25 g each kg ⁻¹ of seed	17.99 (4.24)	32.67 (5.72)	50.66 (7.12)
T ₈ : Control	7.00 (2.64)	13.22 (3.63)	20.22 (4.50)
S.E. $\pm$	1.48	1.88	1.78
CD at 5 %	4.51	5.71	5.41

Figures in parenthesis indicates the square root values

treatment T₂ (24.12). The lowest number of nodules on main root per plant were recorded in treatment T₈ (7.00).

The highest number of nodules on sub root per plant were recorded in treatment T₆ (37.55) and T₂ (37.22) which were at par. The lowest number of nodules on sub root per plant were recorded in treatment T₈ (13.22).

4.5 Leghaemoglobin content in nodules (OD/g)

The data regarding the Leghaemoglobin content of nodules at 60 DAS were presented in Table 9.

The data in Table showed that treatment T₆ (0.544 OD/g nodule) were recorded highest leghaemoglobin content in root nodule followed by T₂ (0.510 OD/g nodule). The lowest leghaemoglobin content were recorded in root nodule of treatment T₈ (0.294 OD/g nodule).

The lignite based treatment i.e. Treatment T₇ was recorded with leghaemoglobin content (0.446 OD/g nodule) which was less as compared to treatment T₆.

4.4.4 Plant height at 30, 60 and 90 days (cm)

The results on plant height recorded at 30, 60 and 90 days were significant and which are presented in Table 10.

The highest plant height at 30 DAS (32.51 cm) was recorded in treatment T₆ followed by treatment T₅ (28.49 cm) and lowest Plant height was recorded in treatment T₈ (21.23 cm). Treatment T₇ recorded with plant height (26.00 cm) which showed the lesser effect on plant height as compared to treatment T₆ and T₅.

Table 9. Leghaemoglobin content (OD/g) at 60 DAS as influenced by various treatments in soybean

Treatment	Leghaemoglobin content (OD/g) of nodule
T ₁ : Liquid <i>Rhizobium</i> 10 ml kg ⁻¹ of seed	0.467
T ₂ : Liquid <i>Rhizobium</i> 25 ml kg ⁻¹ of seed	0.510
T ₃ : Liquid PSB 10 ml kg ⁻¹ of seed	0.324
T ₄ : Liquid PSB 25 ml kg ⁻¹ of seed	0.371
T ₅ : Liquid <i>Rhizobium</i> + PSB @ 10 ml each kg ⁻¹ of seed	0.496
T ₆ : Liquid <i>Rhizobium</i> + PSB @ 25 ml each kg ⁻¹ of seed	0.544
T ₇ : Lignite based <i>Rhizobium</i> + PSB @ 25 g each kg ⁻¹ of seed	0.446
T ₈ : Control	0.294
S.E. $\pm$	0.01
CD at 5 %	0.03

Table 10. Plant height (cm) at 30, 60, 90 days as influenced by various treatments in soybean

Treatment	Plant height (cm)		
	At 30 days	At 60 days	At 90 days
T ₁ : Liquid <i>Rhizobium</i> 10 ml kg ⁻¹ of seed	25.86	52.98	54.44
T ₂ : Liquid <i>Rhizobium</i> 25 ml kg ⁻¹ of seed	27.54	59.21	61.58
T ₃ : Liquid PSB 10 ml kg ⁻¹ of seed	27.12	55.67	57.11
T ₄ : Liquid PSB 25 ml kg ⁻¹ of seed	28.15	59.92	62.20
T ₅ : Liquid <i>Rhizobium</i> + PSB @ 10 ml each kg ⁻¹ of seed	28.49	61.42	63.73
T ₆ : Liquid <i>Rhizobium</i> + PSB @ 25 ml each kg ⁻¹ of seed	32.51	67.35	68.69
T ₇ : Lignite based <i>Rhizobium</i> + PSB @ 25 g each kg ⁻¹ of seed	26.00	58.34	58.49
T ₈ : Control	21.23	47.48	49.10
S.E. $\pm$	0.64	1.55	1.19
CD at 5 %	1.94	4.71	3.60

The maximum plant height at 60 DAS (67.35 cm) was recorded in treatment T₆ followed by T₅ (61.42 cm). The lowest plant height was recorded in treatment T₈ (47.48 cm). Treatment T₇ recorded with plant height (58.34cm) which showed the lesser effect on plant height as compared to treatment T₆ and T₅.

The highest plant height at 90 DAS T₆ (68.69 cm) was recorded in treatment T₆ followed by T₅ (63.73 cm). The lowest plant height was recorded in treatment T₈ (49.10 cm). Treatment T₇ recorded with plant height (58.49 cm) which showed the lesser effect on plant height as compared to treatment T₆ and T₅.

4.7 Days to 50 per cent flowering

The results on days to 50 per cent flowering were statistically significant presented in the Table 11.

The highest days required for 50 per cent flowering (44.50) were recorded in T₈, (44.00) were recorded in treatment T₂ followed by T₇ (43.00). The lowest days required for flowering (38.00) days in treatment T₆ followed by T₅ (39.00), T₄ (40.00) and T₃ (41.00).

4.8 Number of pods per plant

The results in respect of number of pods/plant was presented in Table 11. The results were significant for all the treatments.

Results revealed that treatments T₆ (52.33) consist highest number of pods per plant followed by T₅ (44.66), T₄ (43.67). The lowest number of pods were recorded (22.00) in treatment T₈ followed by T₁ (39.00) and T₇ (40.34).

Table 11. Number of days to 50 per cent flowering, number of pods per plant, 1000 grain weight and grain yield as influenced by various treatments in soybean

Treatment	Number of days to 50 per cent flowering per plant	Number of pods per plant	1000 grain weight (g)	Grain yield (q/ha)
T ₁ : Liquid <i>Rhizobium</i> 10 ml kg ⁻¹ of seed	42.00 (6.48)	39.00 (6.24)	144.67	28.70
T ₂ : Liquid <i>Rhizobium</i> 25 ml kg ⁻¹ of seed	44.00 (6.63)	42.34 (6.50)	146.04	31.36
T ₃ : Liquid PSB 10 ml kg ⁻¹ of seed	41.00 (6.40)	42.00 (6.48)	144.78	25.94
T ₄ : Liquid PSB 25 ml kg ⁻¹ of seed	40.00 (6.32)	43.67 (6.60)	146.25	30.44
T ₅ : Liquid <i>Rhizobium</i> + PSB @ 10 ml each kg ⁻¹ of seed	39.00 (6.24)	44.66 (6.68)	148.10	31.63
T ₆ : Liquid <i>Rhizobium</i> + PSB @ 25 ml each kg ⁻¹ of seed	38.00 (6.16)	52.33 (7.23)	150.23	32.54
T ₇ : Lignite based <i>Rhizobium</i> + PSB @ 25 g each kg ⁻¹ of seed	43.00 (6.56)	40.34 (6.35)	144.42	25.93
T ₈ : Control	44.50 (6.67)	22.00 (4.69)	139.04	23.70
S.E. $\pm$	1.19	2.77	1.23	1.37
CD at 5 %	3.60	8.40	3.73	4.15

Figures in parenthesis indicates the square root values

4.9 1000 grain weight in gram

The data in the Table 11 revealed that the highest 1000 seed weight (150.23 g) was observed in treatment T₆ followed by T₅ (148.10 g). The 1000 seed count of lignite based treatment T₇ (144.42 g) was less than combined liquid biofertilizer treatment i.e. T₆ and T₅. The least 1000 grain weight was observed in treatment T₈ (139.04 g).

4.10 Grain yield (q/ha)

The data regarding the grain yield is presented in the Table 11.

The maximum grain yield (32.54 q/ha) was produced in the treatment T₆ followed by T₅ (31.63 q/ha) and the least (23.70 q/ha) was recorded in the treatment T₈. The yield of combined effect of liquid biofertilizer treatment T₆ and T₅ and also alone liquid biofertilizer i.e. T₄ (30.44 q/ha), T₂ (31.36 q/ha) are significantly superior over lignite based treatment T₇ (25.93 q/ha). All the results were found to be statistically significant.

5. DISCUSSION

Field experiment entitled “Studies on seed inoculation of MPKV’s *Rhizobium* and liquid biofertilizer in soybean” was conducted during *kharif* 2013 at the field of Department of Plant Pathology and Agricultural Microbiology, Mahatma Phule Krishi Vidyapeeth, Rahuri (Ahmednagar). The results reported in the previous chapter are discussed here under.

5.1 *Rhizobium* count

The results (Table 1) in general revealed that all the inoculation treatments including even the *Rhizobium* alone significantly enhance the *Rhizobium* count over non inoculated treatments. Combined effect of liquid *Rhizobium* biofertilizer with PSB liquid biofertilizer inoculated treatments significantly enhanced the *Rhizobium* count which clearly indicated the symbiotic association of *Rhizobium* with PSB with maximum *Rhizobium* count recorded at 60 DAS. These results were in close agreement with *Rhizobium* inoculation along with PSB has been reported to increase the *Rhizobium* count of chickpea (Algawadi and Gaur, 1988).

The effect of liquid biofertilizer in combination and *Rhizobium* inoculation alone also reported increased the *Rhizobium* count over lignite based inoculation. These results were in close agreement with increase in *Azotobacter* count due

to the inoculation of maize with liquid *Azotobacter* culture than lignite based *Azotobacter* culture (Mane, 2011).

5.2 PSB count

The results (Table 2) revealed that inoculation of *Rhizobium* + PSB liquid biofertilizer treatments were recorded maximum PSB count. These results were in close agreement with increase in PSB count due to *Rhizobium* has been reported in gram by (Swarnalakshmi, 2006). The single PSB liquid biofertilizer treatments also increased PSB count over lignite based and uninoculated control with maximum PSB count recorded at 60 DAS. These results were in close agreement with PSB inoculation increased PSB count reported by Singh *et al.*, (2005) and Nahas *et al.* (1994), Kim *et al.* (1998) and Gupta (2012).

5.3 Nodulation pattern

The number of soybean root nodules (Table 4,5,6,7,8) were maximum at 60 DAS this results were in close agreement with (Chaudhary *et al.*, 1983). Increased number of nodules were observed with combined liquid biofertilizer inoculation treatments (*Rhizobium* + PSB @ 25 ml each kg⁻¹ of seed). Same trend was recorded by Sheelavantar (1982), Hegde (2001) and Tran Thi *et al.* (2007).

The results regarding the number of nodules were indicated that the number of white nodules were maximum at 30 DAS which showed the initial microbial activities whereas at 60 DAS number of pink nodules were recorded with highest

count which indicates the active microbial activities with high leghaemoglobin content. At 90 DAS more number of black nodules were recorded which indicates less microbial activities these result was in close agreement with (Maiti and Sen, 1990). The number of nodulations were more on sub root as compared to main root. The present result of increased root nodules and pigmentation of root nodules of soybean crop are in close agreement with Shrivastava and Ahlawat (1995), Hunt *et al.* (1985), Katiyar and Pant (1993), Pant *et al.* (1995).

5.4 Leghaemoglobin content in nodules

It was found from the data (Table 9) that the leghaemoglobin content in soybean root nodules (Pink root nodules) at 60 DAS was higher in all the inoculated treatments over uninoculated control. Further it was also noted that the dual inoculation of *Rhizobium* + PSB liquid biofertilizer treatments recorded maximum leghaemoglobin content. Same results were recorded by Algawadi and Gaur (1988) in case of gram. Followed by single inoculation of *Rhizobium* liquid biofertilizer treatments which clearly reflects the high nitrogen fixing ability of symbiotic microorganisms.

Same trends were recorded by Tagore *et al.* (2013) in case of chickpea.

5.5 Plant height

The results (Table 10) in general indicated that the inoculation treatments recorded significantly more plant height amongst them the combined inoculation treatment (*Rhizobium* +

PSB) recorded maximum plant height than their single inoculations at all the stages of crop growth. These results were in close agreement with (Shrivastava and Ahlawat, 1995).

The inoculation of *Rhizobium* has been reported to increase the plant height in case of soybean (Jacob and Dugue, 1982 and Singh *et al.* 1987). The inoculation with liquid *Rhizobium* biofertilizer treatment and combined treatments (*Rhizobium* + PSB) showed better result than carrier based (lignit based) biofertilizer inoculation on treatment same trends were recorded by Singaravel *et al.* (2008) in case of okra and by Hegde (2002) in field trial of soybean, pigeonpea and groundnut.

5.6 Days to 50 per cent flowering

The results (Table 11) in general indicated that the inoculation treatments recorded significantly less days to flowering in case of soybean crop as compare to uninoculated treatment. Amongst them the combined inoculation treatments requires less days to 50 per cent flowering as compared to single inoculation treatments and lignite based these results were in close agreement with Pant *et al.* (1995) and Hynes *et al.* (1995).

Also the single PSB liquid biofertilizer inoculation treatment was recorded less days to 50 per cent flowering than *Rhizobium* liquid biofertilizer inoculation treatment. These results were in close agreement with Mehta *et al.* (1995).

5.7 Number of pods per plant

The results (Table 11) in general indicated that the inoculation treatments recorded significantly more number of

Pods per plant of soybean as compared to uninoculated treatment. Amongst them the combined (*Rhizobium* + PSB) liquid biofertilizer inoculation treatment recorded maximum pods per plant as compared to single inoculated and lignite based inoculation treatment. Same trend was recorded by Hegde (2002) in case of field trial of soybean, pigeonpea and groundnut and by Shivkumar (1998) in case of chickpea.

Also the single PSB liquid biofertilizer inoculation treatment was recorded high number of pods per plant. These results were in close agreement with results demonstrated by Kamparia (1995) in case of soybean, groundnut and mung.

5.8 1000 grain weight

The results (Table 11) pertaining to 1000 grain weight of soybean seed showed increased weights with combined liquid biofertilizer inoculation treatment (*Rhizobium* + PSB) as compared to lignite based biofertilizer inoculation treatment.

These results were in close agreement with the results of (Mane, 2011) in case of maize and Pant *et al.* (1995).

Also the single *Rhizobium* liquid biofertilizer inoculation treatment and PSB liquid biofertilizer treatment were recorded with high 1000 grain weight. These results were in close agreement with the results demonstrated by (Sable and Khuspe 1977) in case of soybean and (Kushadev, 1956) in case of wheat, maize and soybean.

5.9 Grain yield

The results (Table 11) of grain yield were found to be increased with single and dual inoculation of *Rhizobium* and PSB liquid biofertilizer as compared to uninoculated soybean seed. Amongst them the combined liquid biofertilizer inoculation treatments (*Rhizobium* + PSB) were recorded highest grain yield as compared to lignite based biofertilizer treatment.

Same trends were recorded by (Natrajan *et al.* 1980) in case of French beans and by (Mane 2011) recorded effective use of liquid *Azotobacter* culture on maize crop over lignite based *Azotobacter* culture.

Also the single use of liquid biofertilizers like *Rhizoibum* and PSB shows better result in case of grain yield of soybean crop as compared to lignite based biofertilizer treatment.

These results were in close agreement with the results stated by Rewari *et al.* (1973), Kushadev (1956), Tran Thi *et al.* (2007), Singaravel *et al.* (2008) and Hegde (2002).

6. SUMMARY AND CONCLUSIONS

The present investigation entitled “Studies on seed inoculation of MPKV’s *Rhizobium* and PSB liquid biofertilizer in soybean was conducted at the field of Department of Plant Pathology and Agril. Microbiology in kharif season of year 2013-14 was planned with an aim to study the effect of MPKV’S liquid biofertilizer *Rhizobium* and PSB on growth and yield of soybean crop by standardizing the dose of *Rhizobium* and PSB liquid biofertilizer.

The observations were recorded on various growth parameters of soybean crop viz., plant height, number of pods per plant, number of nodules, days to 50 per cent flowering, 1000 grain weight, grain yield, microbial population at different stages of crop growth and leghaemoglobin content of soybean root nodules.

Thus from the results of present research work it can be concluded that,

1. The *Rhizobium* and PSB population were recorded higher in case of seed inoculation of MPKV’S liquid biofertilizers as compared with lignite based biofertilizer inoculation and uninoculated treatment.
2. Inoculation with MPKV’s liquid biofertilizer *Rhizobium* + PSB @ 25 ml each kg⁻¹ of seed had profound effect on all growth parameters. In short, the combined effect of liquid biofertilizer inoculation (*Rhizobium* + PSB) showed maximum beneficial effect on different growth parameters of soybean crop.

3. Seed inoculation of soybean with MPKV's *Rhizobium* + PSB liquid biofertilizers showed the better result over the seed inoculation with MPKV'S lignite based biofertilizers.
4. The number of root nodules were recorded more in case of combined (*Rhizobium* + PSB)liquid biofertilizer inoculation treatment as compared with single inoculation of liquid biofertilizer, lignite based biofertilizer inoculation and uninoculated/control treatment.
5. The higher values of microbial population, plant height, number of pods per plant, number of nodules and leghaemoglobin content were recorded at 60 DAS of soybean crop growth which clearly reflects the higher activities of *Rhizobium* and *PSB* microorganisms at 60 DAS of crop growth.
6. The finding explicitly indicated the possibility of saving fertilizer N, P, K the extent of 25 percent.
7. The inoculation of soybean seed with *Rhizobium* and PSB liquid biofertilizer recorded the increase in yield of soybean upto 37 per cent.

Considering all the observations recorded during the field and laboratory investigations it can be clearly concluded that the seed inoculation with MPKV'S *Rhizobium* and PSB liquid biofertilizers @ 25 ml each kg⁻¹ of seed showed significant effect on nodulation, growth and yield of soybean crop.

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8. APPENDIX

a. Congo Red Yeast Extract Manital Agar Medium (CRYEMA)

- | | | | |
|-----|-------------------------------------|---|---------|
| 1. | Mannitol | : | 10.0 gm |
| 2. | KH ₂ PO ₄ | : | 0.5 gm |
| 3. | MgSO ₄ 7H ₂ O | : | 0.2 gm |
| 4. | NaCl | : | 0.1 gm |
| 5. | CaCO ₃ | : | 3.0 gm |
| 6. | Congo red (1%) | : | 2.5 ml |
| 7. | Yeast extract | : | 1 gm |
| 8. | Agar Agar | : | 20 gm |
| 9. | Distilled water | : | 1000 ml |
| 10. | pH | : | 7 |

b. Pikovaskaya's media for Phosphate solubilizing microorganism

- | | | | |
|-----|---|---|-----------|
| 1. | Glucose | : | 10 g |
| 2. | Ca ₃ (PO ₄) ₂ | : | 5 g |
| 3. | (NH ₄) ₂ SO ₄ | : | 0.5 g |
| 4. | KCl | : | 0.2 g |
| 5. | MgSO ₄ , H ₂ O | : | 0.1 g |
| 6. | MnSO ₄ | : | Trace |
| 7. | FeSO ₄ | : | Trace |
| 8. | Yeast extract | : | 0.5 g |
| 9. | Agar | : | 15 g |
| 10. | Distilled water | : | 1000 ml |
| 11. | pH | : | 7.0 - 7.2 |

9. VITA

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of
MASTER OF SCIENCE (AGRICULTURE)
in
AGRICULTURAL MICROBIOLOGY
2014

Title of thesis : “Studies on seed inoculation of MPKV’s Rhizobium and PSB liquid Biofertilizer in Soybean”

Major field : Agricultural Microbiology

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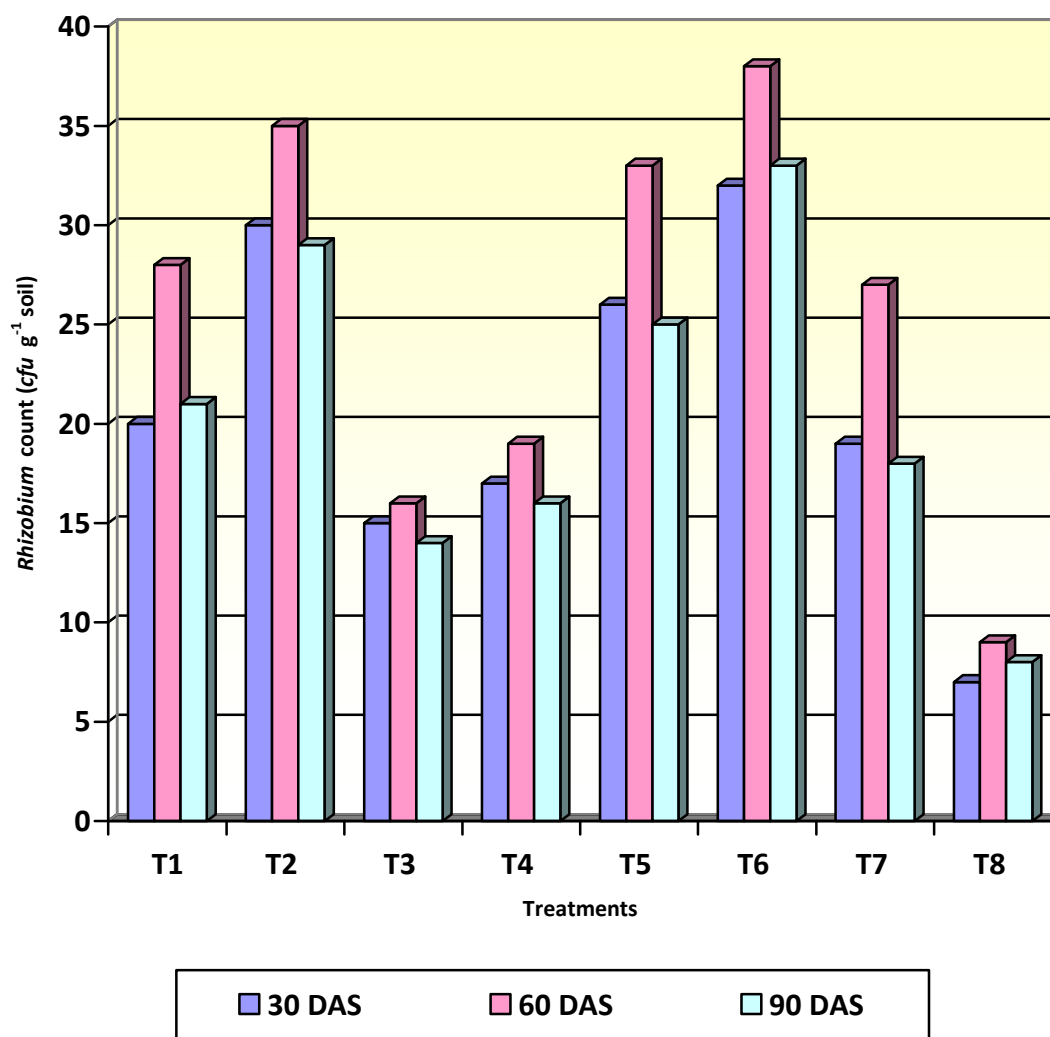


Fig. 1. *Rhizobium* count at 30, 60, 90 DAS at 10^{-5} dilution (cfu g⁻¹ soil) as influenced by various treatments in soybean

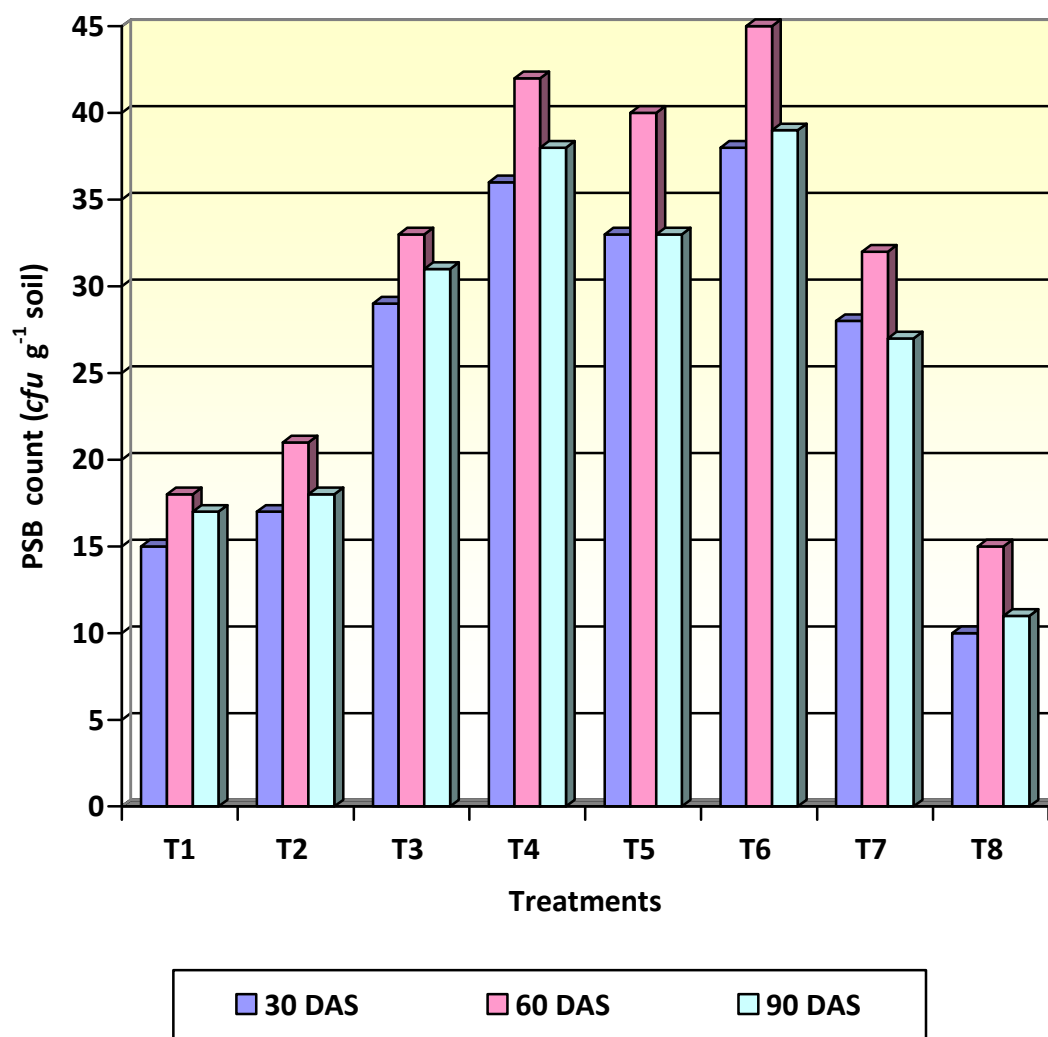


Fig. 2. PSB count at 30, 60 , 90 DAS at 10⁻⁵ dilution (cfu g⁻¹ soil) as influenced by various treatments in soybean

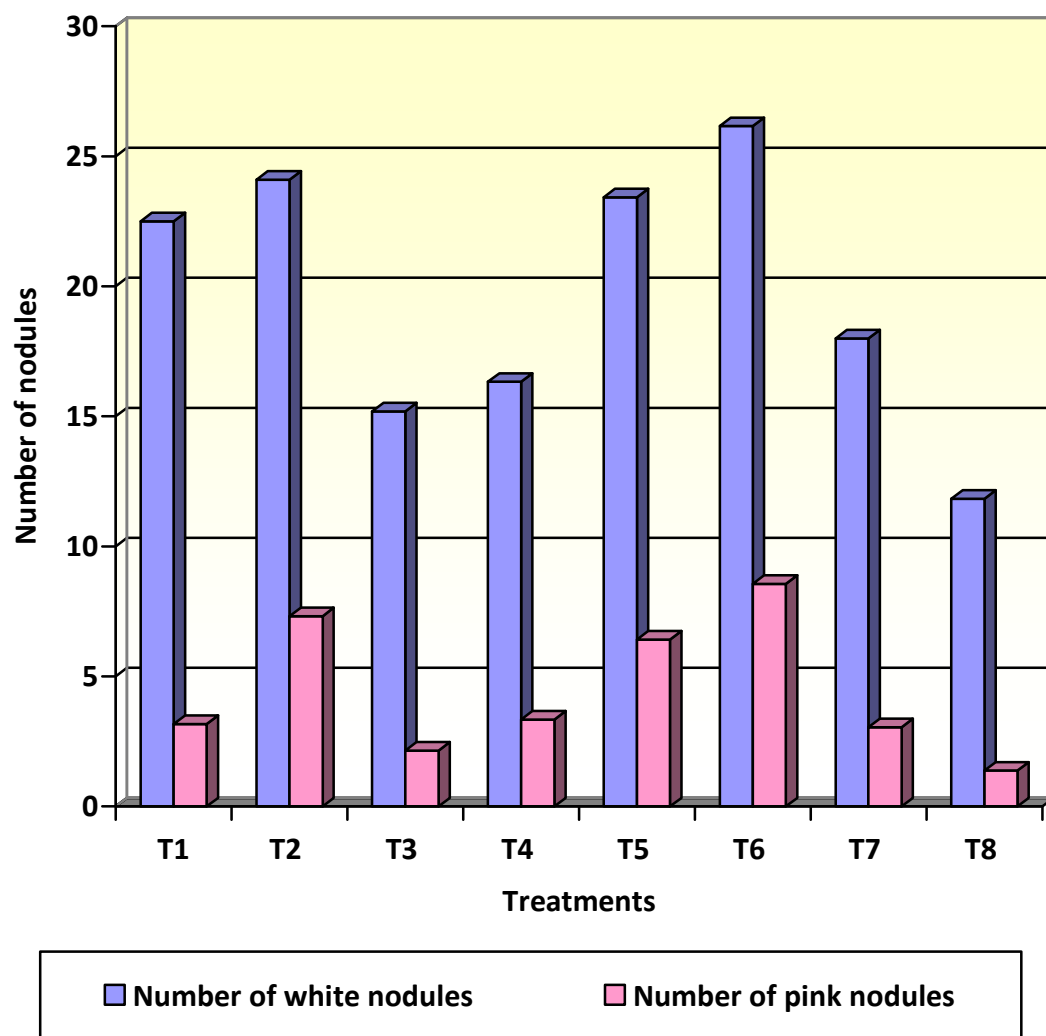


Fig. 3. Number of nodules plant⁻¹: white, pink and black nodules at 30 DAS as influenced by various treatments in soybean

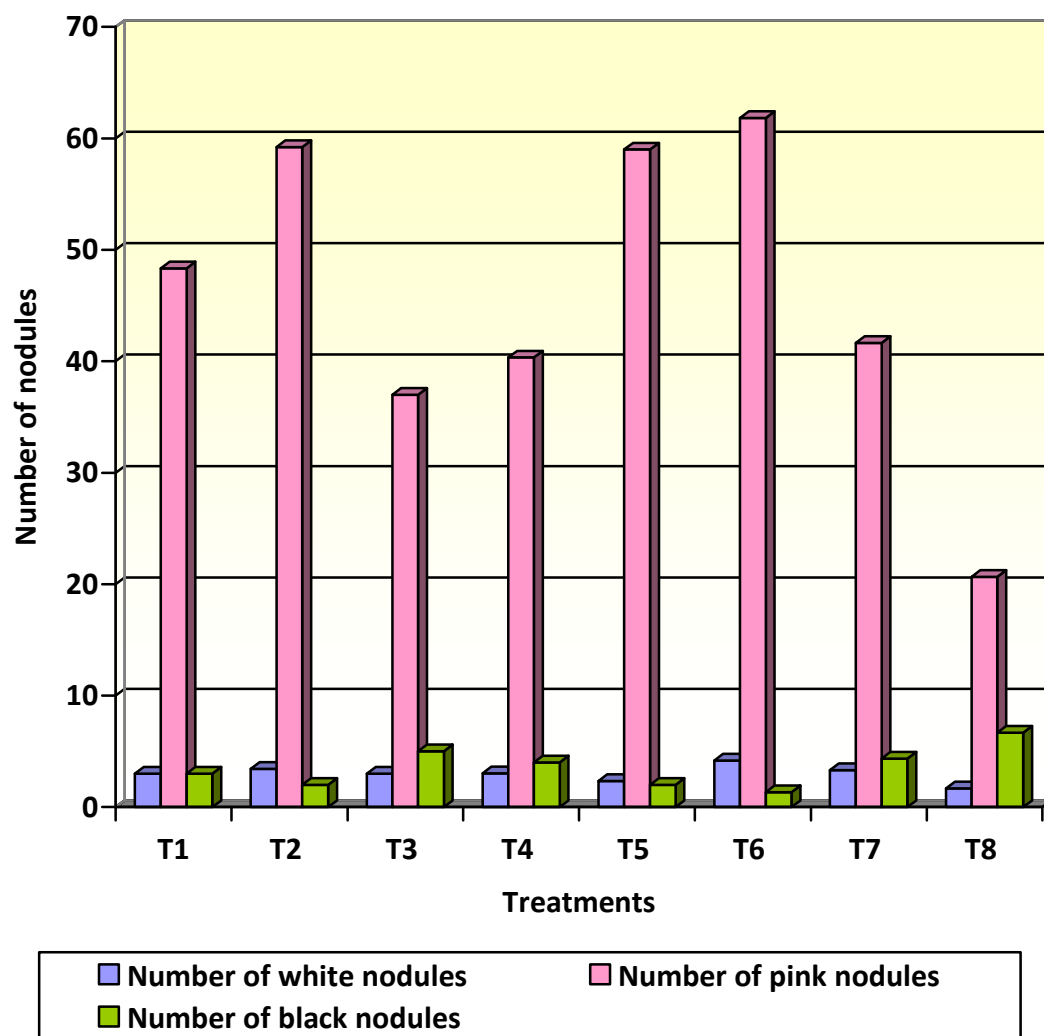


Fig. 4. Number of nodules plant⁻¹: white, pink and black nodules at 60 DAS as influenced by various treatments in soybean

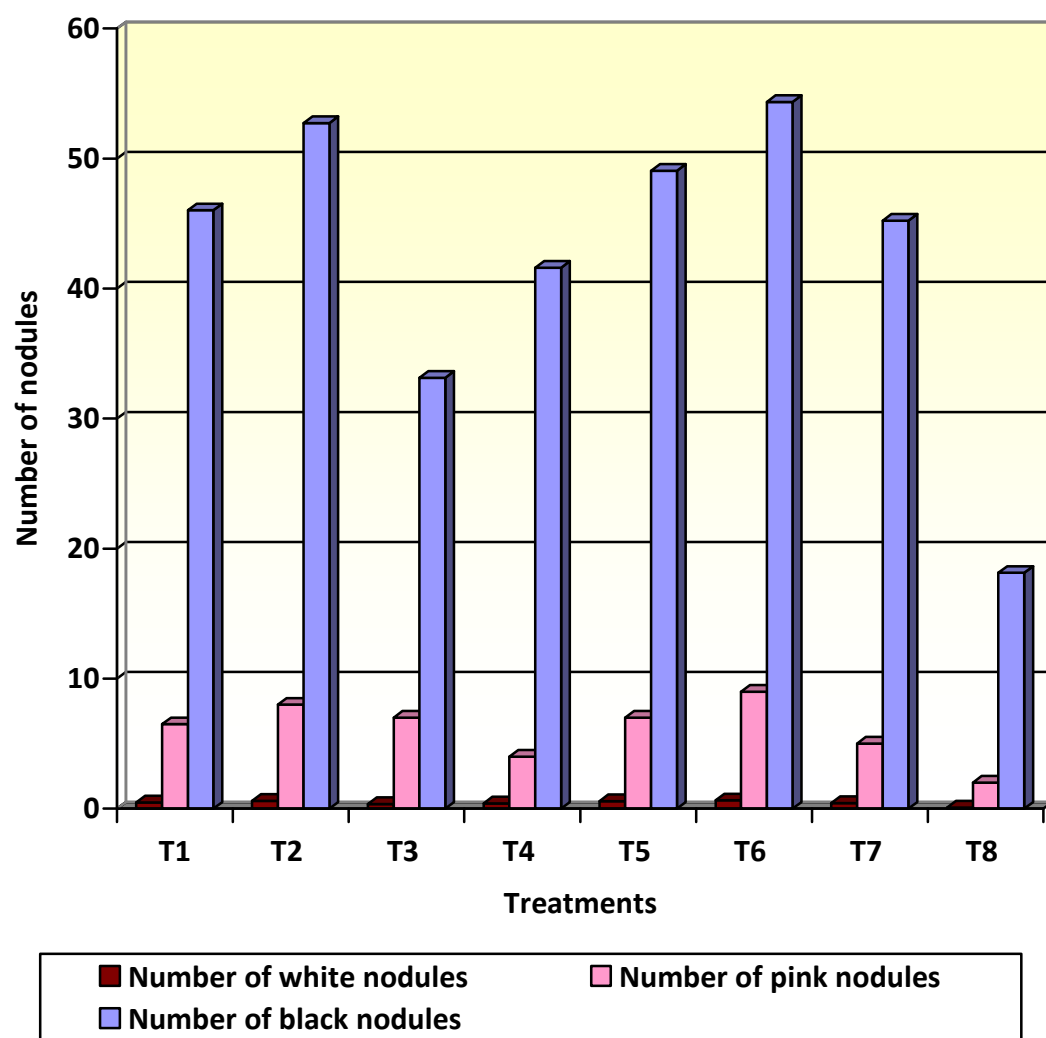


Fig. 5. Number of nodules plant⁻¹: white, pink and black nodules at 90 DAS as influenced by various treatments in soybean

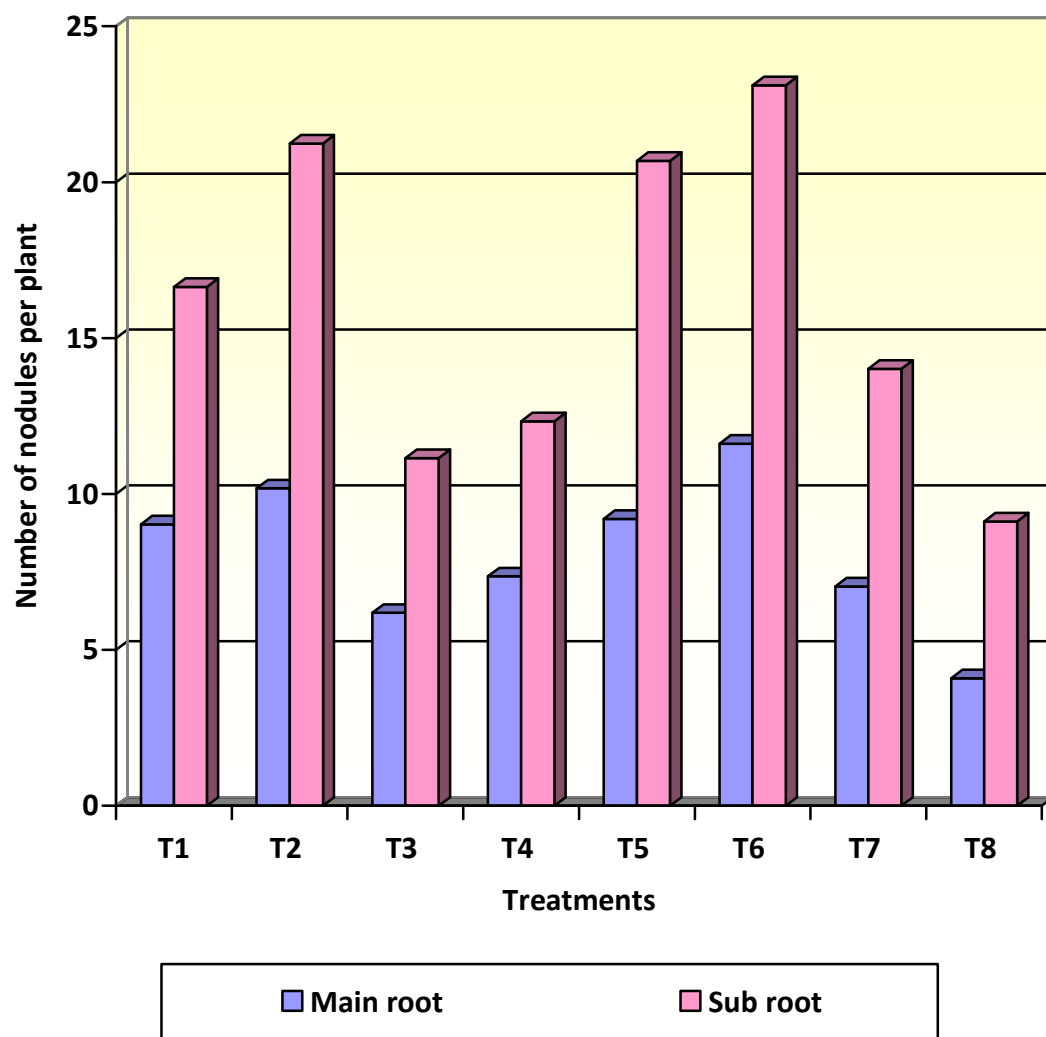


Fig. 6. Number of nodules plant⁻¹ on main root and sub root at 30 DAS as influenced by various treatments in soybean

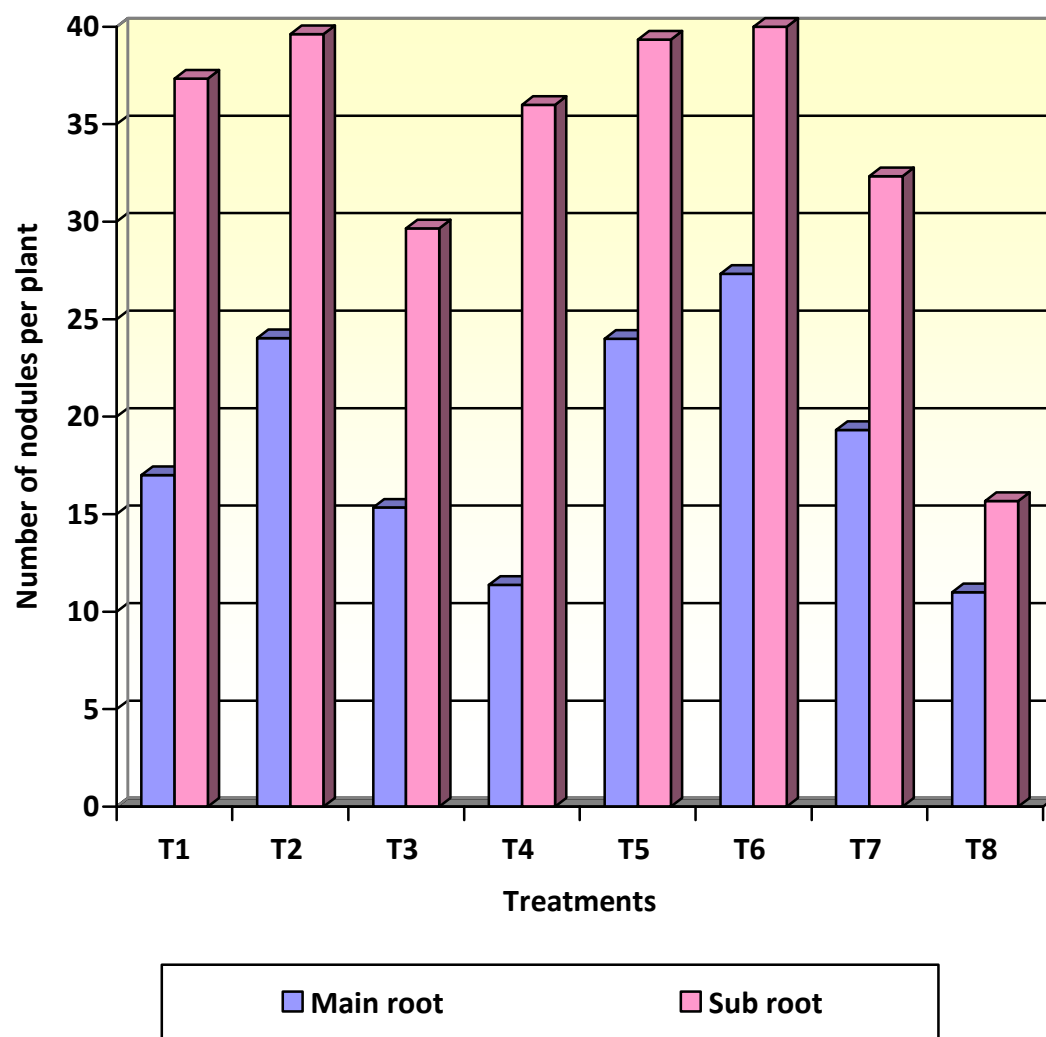


Fig. 7. Number of nodules plant⁻¹ on main root and sub root at 60 DAS as influenced by various treatments in soybean

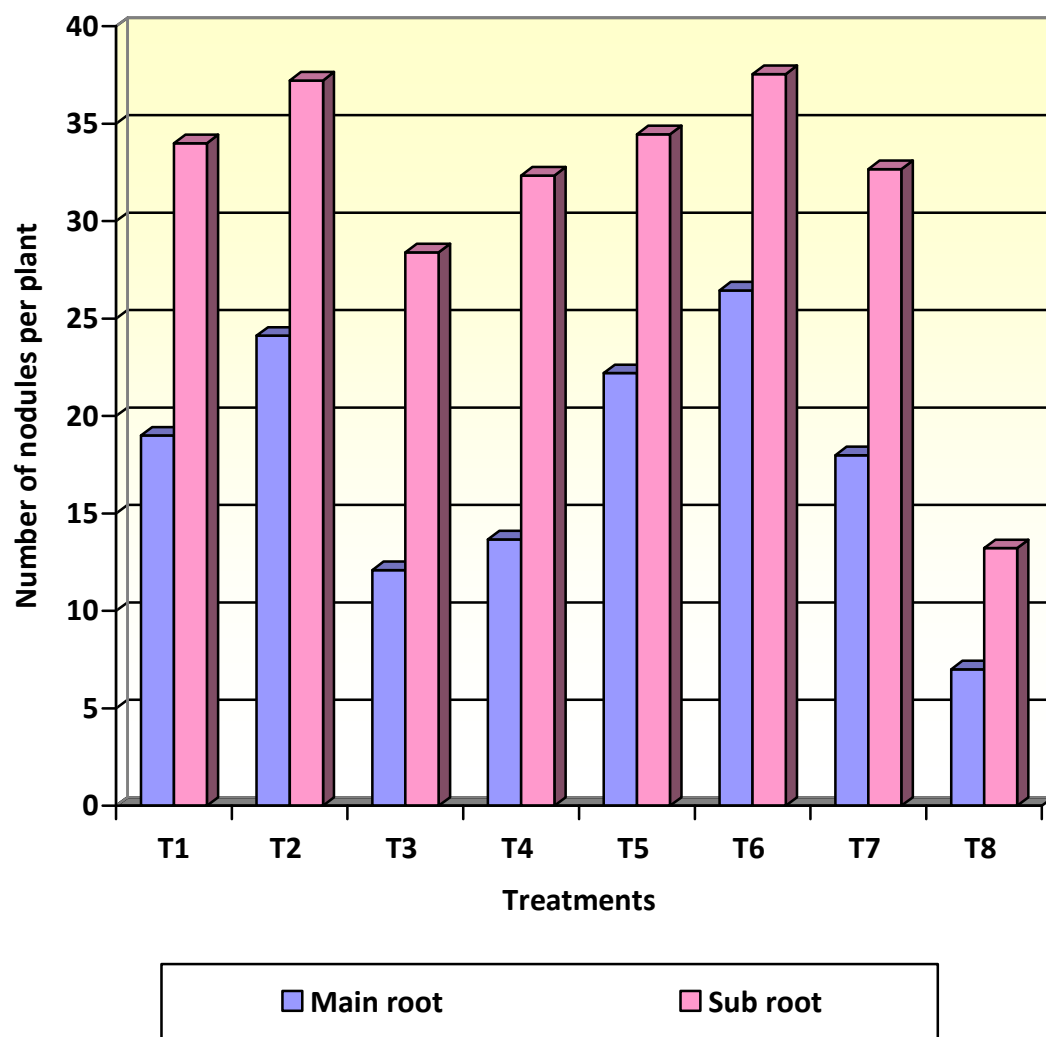


Fig. 8. Number of nodules plant⁻¹ on main root and sub root at 90 DAS as influenced by various treatments in soybean

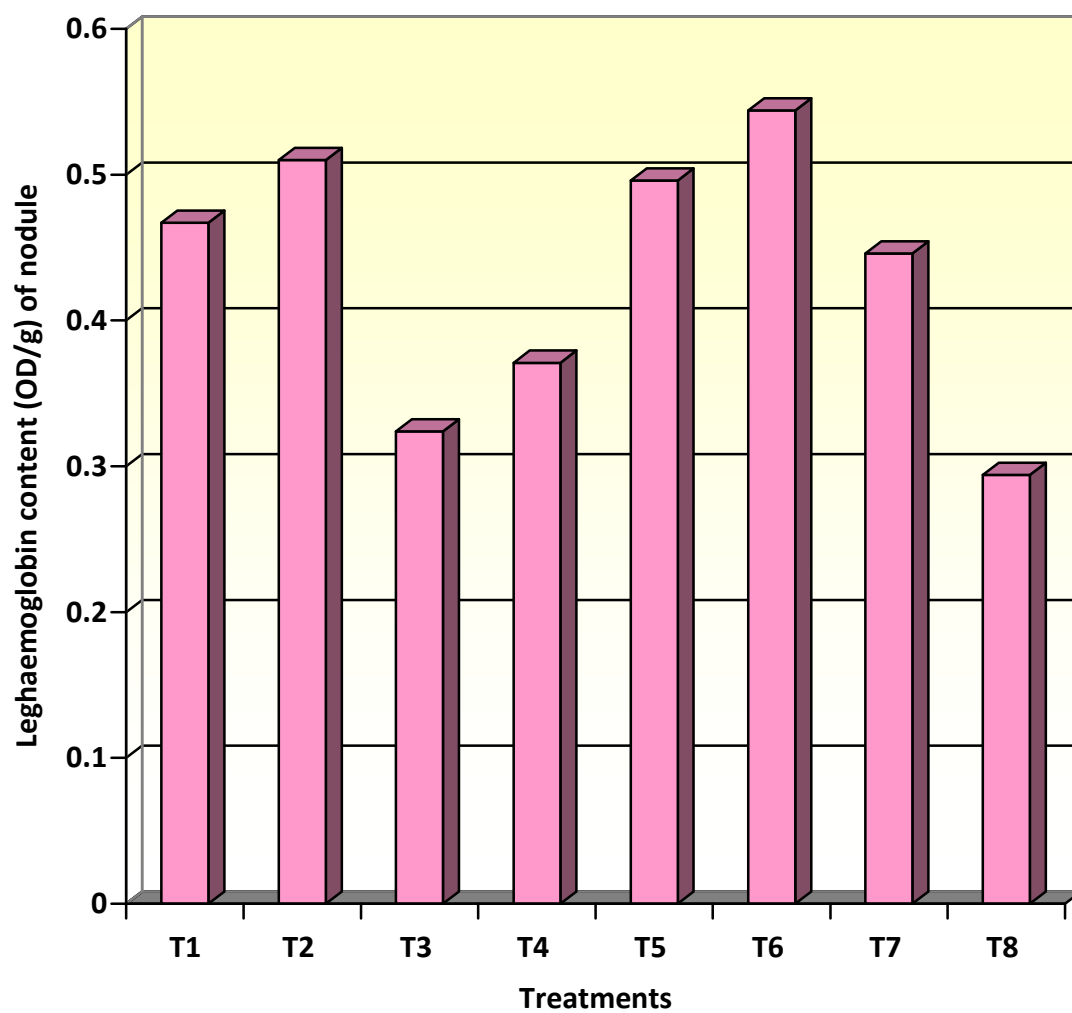


Fig. 9. Leghaemoglobin content (OD/g) at 60 DAS as influenced by various treatments in soybean

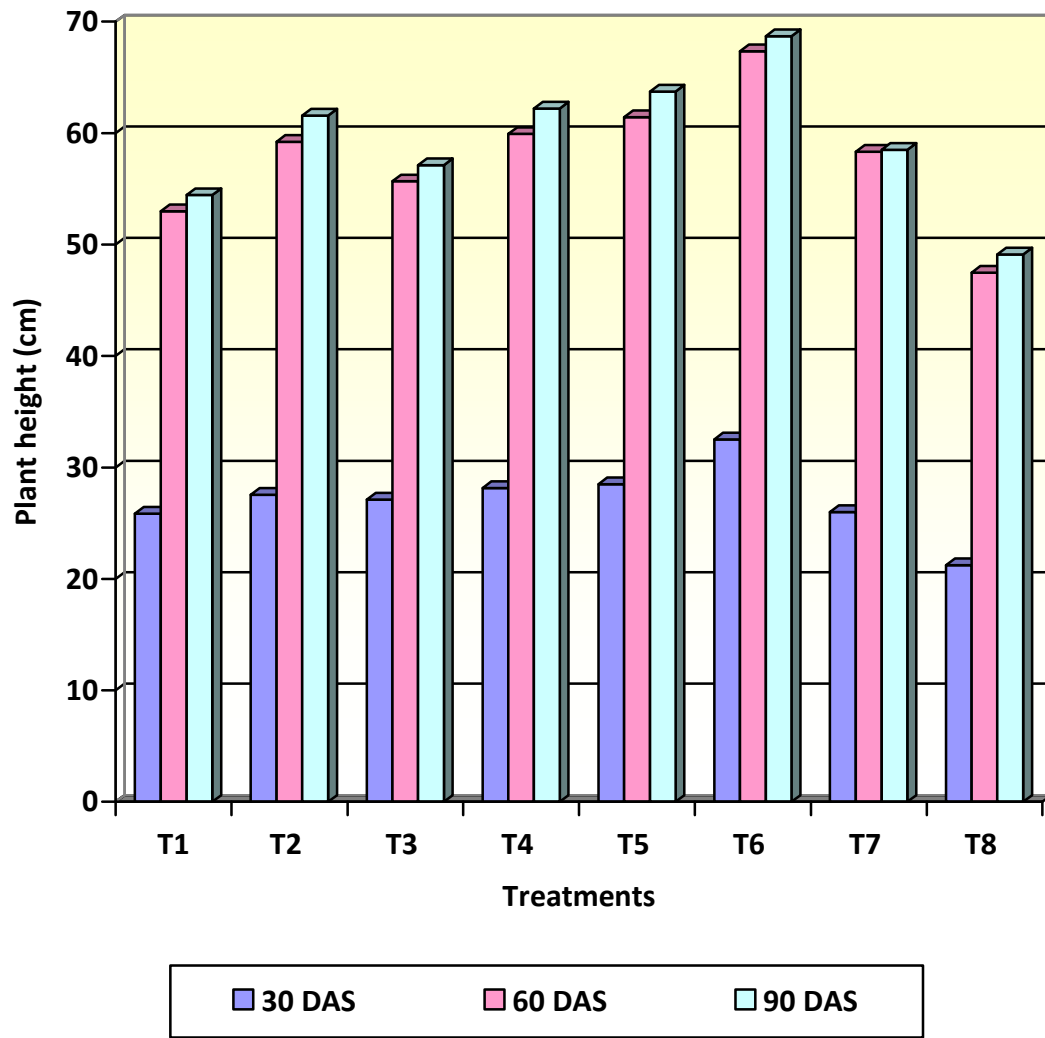


Fig. 10. Plant height (cm) at 30, 60, 90 days as influenced by various treatments in soybean

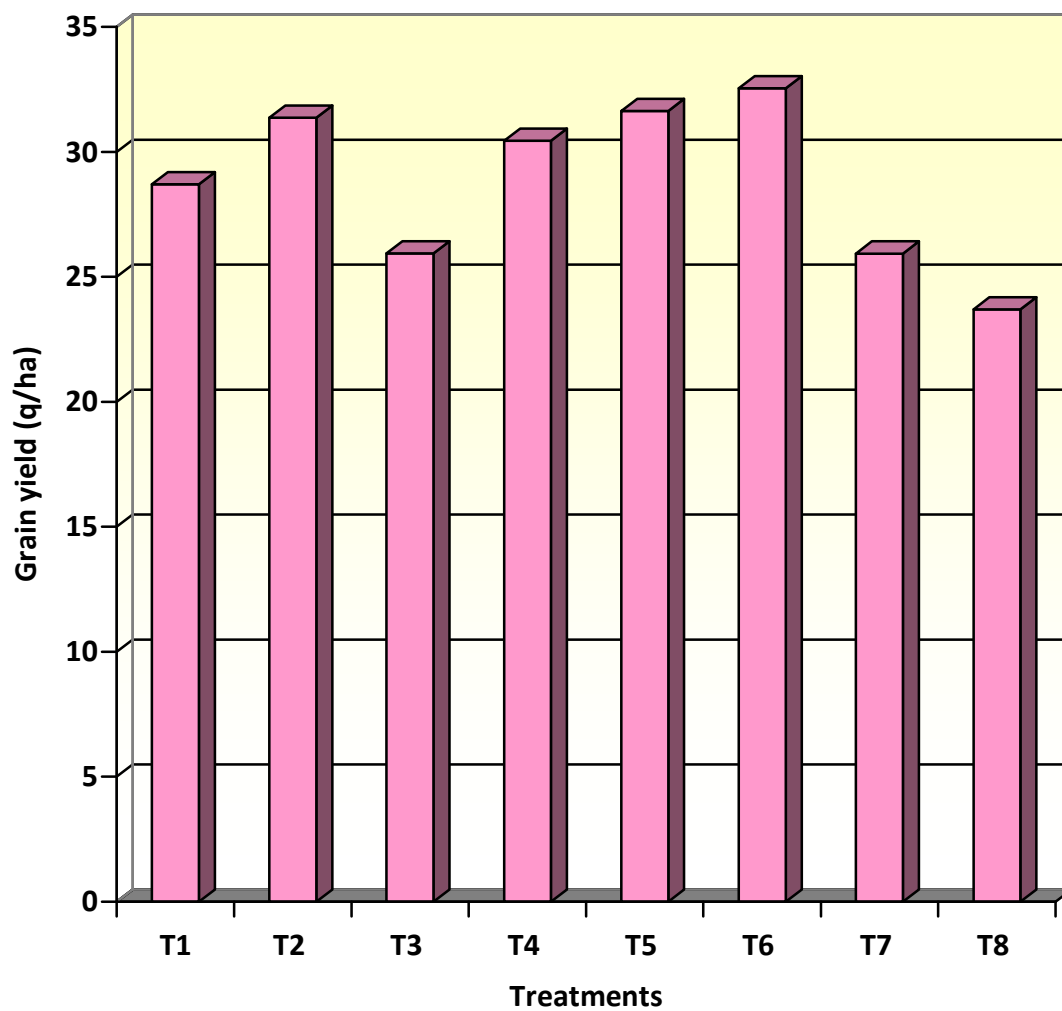


Fig. 11. Grain yield (q/ha) as influenced by various treatments in soybean